PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02, C07K 5/00, 7/00, 15/00, 17/00

A1

(11) International Publication Number: WO 95/00161

(43) International Publication Date: 5 January 1995 (05.01.95)

(21) International Application Number: PCT/US94/06837

(22) International Filing Date: 16 June 1994 (16.06.94)

(30) Priority Data:
08/079,319 18 June 1993 (18.06.93) US

(71) Applicant (*for all designated States except US*): UNIVERSITY OF CINCINNATI [US/US]; Mall Location 627, Cincinnati, OH 45221-0726 (US).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): BALASUBRAMANIAM, Ambikaipakan [US/US]; 2706 Lawyers Pointe Drive, Cincinnati, OH 45244 (US).

(74) Agent: CLARK, Paul, T.; Fish and Richardson, 225 Franklin Street, Boston, MA 02110 (US).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NEUROPEPTIDE Y ANTAGONISTS AND AGONISTS

(57) Abstract

The invention discloses analogs which behave as NPY antagonists and agonists; and methods of their use for controlling a biological activity such as appetite and cardiovascular function.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgystan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

NEUROPEPTIDE Y ANTAGONISTS AND AGONISTS

Background of the Invention

This invention relates to peptide derivatives which are antagonists or agonists of neuropeptide Y.

Neuropeptide Y (NPY), is a 36-residue peptide characterized by a tyrosine (Y) residue at its N-terminus and a tyrosine amide residue at its C-terminus. The peptide was isolated from porcine brain (Tatemoto *Proc. Natl. Acad. Sci.* U.S.A. 79:5485-5489, 1982) and is considered to be a neurotransmitter or neuromodulator widely distributed in the central and peripheral nervous systems (Allen et al., *Neurochem. Int.* 8:1-8, 1986). It is the most abundant peptide present in the mammalian brain and heart (Edvinsson et al., *Trends Pharmol. Sci.* 8:231-235, 1987; Gu et al., *Histochem. Cytochem.* 32:467-472, 1984), and is among the most potent vasoconstrictor peptides isolated to date (Lundberg et al., *Acta Physiol. Scand.* 121:325-332, 1984). However, NPY elicits several physiological responses by activating specific pre- and post-synaptic receptors. Centrally, NPY is thought to be involved in the regulation of food intake, memory processing and circadian rhythm (Sheikh et al., *FEBS Lett.* 245: 209-214, 1989). In the periphery, NPY seems to function as a transmitter in sympathetic nerves where it interacts with norepinephrine mainly in the regulation of vasculartone (Sheikh et al. *FEBS Lett.* 245:209-214, 1989).

Different structure-activity relationships for NPY analogs in various model systems have indicated that multiple NPY receptor subtypes exist (Michel, *Tips* 12:389-394, 1991). Wahlestedt and coworkers (*Regul. Pept.* 13:307-318, 1986) first suggested the existence of two distinct subtypes of NPY receptors. Post-synaptic (Y1-type) effects could be obtained with the complete NPY

molecule, while pre-synaptic (Y2-type) effects were found elicited by long C-terminal fragments, as well as with the entire NPY molecule. Thus, both Y1 and Y2 receptors exhibit nearly equal affinity to NPY and its homologous peptide, peptide YY, but only the Y2 receptors could bind to shorter carboxyl-terminal fragments including NPY(13-36) as described by Sheikh et al. (*FEBS Lett*. 245:209-214, 1989). However, since NPY receptors in rat cardiac ventricular membranes discriminate between NPY and peptide YY but bind NPY(13-36), it was suggested that this system be classified as a subtype of Y2 or a new class (designated Y3) of receptors as discussed below (Balasubramaniam et al. *Peptides* 11:545-550, 1990).

NPY is also present in high concentrations in a distinct population of nerve fibers innervating the heart and blood vessels (Wharton et al., *Ann. N.Y. Acad. Sci.* 611:133-144, 1990). NPY is now regarded as the predominant peptide present in the cardiovascular system of mammals. This observation has led to numerous studies of the cardiovascular properties of NPY. For example, several investigations have reported that NPY is a potent vasopressor peptide and that it inhibits the coronary blood flow and contractility in isolated perfused hearts (e.g., see Balasubramaniam et al., *Regul. Pept*. 21:289-299, 1988; Allen et al. *Regul. Pept*. 6:247-253, 1983; Rioux et al. *Peptides* 7:27-31, 1986). Furthermore, NPY is also capable of (1) inhibiting the contractile force of isolated cardiac muscles (Balasubramaniam et al. *supra*) and myocytes (Piper et al. *Nuanyn-Scniedberg's Arch. Pharmol*. 340: 333-337, 1989) and (2) the adenylate cyclase activity and cAMP production by cardiac muscles (Kassis et al., J. Biol. Chem. 262: 3429-3431, 1987) and myocytes (Kassis et al.*supra*; Millar et al. *Nuanyn-Scniedberg's Arch. Pharmol*. 338:426-429, 1989), respectively. Specific receptors of NPY in rat cardiac

ventricular membranes have been characterized and reported to be more selective to NPY than previously characterized NPY receptors as discussed above (Balasubramaniam et al. *Peptides* 11:545-550, 1990). The presence of specific receptors of NPY in rat cardiac membranes, the Y-3 receptor, was also reported by visualization with N^{α} biotinyl-NPY analogs (Balasubramaniam et al. *Peptides* 11: 1151-1155, 1990).

The following table (the abbreviations used are commonly known in the art and are described *infra*) shows the amino acid homology between NPY and PYY:

```
                  5    10   15   20   25   30   35
              ----+----+----+----+----+----+----+-
Human NPY     YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Rat NPY       YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Rabbit NPY    YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Guinea pig NPY YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Porcine NPY   YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY

Human PYY     YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY
Porcine PYY   --A--------------S------------------
Rat PYY       --A--------------S------------------
```

NPY has been implicated in the pathophysiology of a number of diseases including, without limitation, obesity, hypertension and chronic heart failure (CHF) because: (1) hypothalamic NPY levels are elevated in obese rats and decreased in cancer anorectic rats; (2) plasma NPY levels are elevated in CHF and hypertensive patients; (3) negative cardiac inotropic and chronotropic actions; and (4) inhibition of libido and circadian rhythm. Thus, since NPY has been shown to be important for regulating a plurality of physiological events we have set out to design a series of receptor-specific analogs that selectively modulate a variety of biological activities, e.g., appetite and blood pressure activities.

Summary of the Invention

In general, the invention features analogs which behave as NPY antagonists and agonists.

In one aspect, the present invention features compounds having the formula:

$$\begin{array}{l} R_1 \\ \quad\diagdown \\ R_2 - A^1\text{-}A^2\text{-}A^3\text{-}A^4\text{-}A^5\text{-}A^6\text{-}Y\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}A^{33}\text{-} \\ \qquad\qquad\qquad\qquad A^{34}\text{-}A^{35}\text{-}A^{36} - W \qquad\qquad (I) \end{array}$$

wherein each

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl (e.g.,methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

A^1 is Tyr, or any aromatic amino acid;

A^2 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal or Asp;

A^3 is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;

A^4 is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^5 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, or D-Trp;

A^6 is Gly or is the D- or L- isomer selected from the group consisting of Asp, Glu, N-Me-Asp, Ala, or Aoc;

Y is A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-A^{24} or is absent, where

A^7 is Asn, Ala, Gln, Gly, or N-Me-Asn;

A^8 is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac_6c, or D-Pal;

A^9 is Gly, N-Me-Gly, Ala, or Trp;

A^{10} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{11} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{12} is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A^{13} is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A^{14} is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A^{15} is Glu, Asp, N-Me-Glu, Ala, or Nva;
A^{16} is Asp, Glu, N-Me-Asp, Ala, or Anb;
A^{17} is Met, Leu, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;
A^{18} is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;
A^{19} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;
A^{20} is Tyr, or any aromatic amino acid;
A^{21} is Tyr, or any aromatic amino acid;
A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala,
A^{23} is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, N-Me-Ser, or N-Me-Thr;
A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;
A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotroptophan, or Tcc);

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic amino acid;

W is -OH, -N-R_3R_4, or OR_5 (where R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl, (e.g., p-methylphenyl); wherein, in formula (I) each bond can represent either a peptide

bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

Preferably, said pseudopeptide bond is between amino acid residues A^{29}-A^{30}, A^{34}-A^{35}, and A^{35}-A^{36}.

Preferred compounds formula (I) include those in which A^{32} is D-Trp, D-Phe, D-Tyr, D-Bip, D-Dip, D-Bth, D-Nal, 2-Cl-Trp, Tcc, Trp, or a pharmaceutically acceptable salt thereof. In yet other preferred embodiments of the invention the compounds of formula (I) include those in which Y (A^7-A^{24}) is deleted. Preferably, the compound of formula (I) is [D-Trp^{32}]NPY, cyclo (2/27) Des-AA$^{7-24}$[Asp^2, D-Ala^6, D-Lys^{27}, D-Trp^{32}]NPY, Des-AA$^{7-24}$[D-Ala^5, Aoc^6, D-Trp^{32}]NPY, Des-AA$^{7-24}$[D-Ala^5, Gly^6, D-Trp^{32}]NPY or Des-AA$^{7-24}$[D-Trp^5, Aoc^6, D-Trp^{32}]NPY .

In another aspect, the invention features a compound having the formula:

$$\begin{matrix} R_1 \diagdown \\ R_2 - X\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36}\text{-}\ W \end{matrix} \qquad \text{(II)}$$

wherein X is a chain of 0-7 amino acids, inclusive the N-terminal one of which is bonded to each R_1 and R_2; wherein each R_1 and R_2, independently, is

H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu, or is deleted;

A^{31} is Ile, Cys, D-Ala, Leu, Val, Aib, Anb, N-Me-Ile, or is deleted;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotroptophan, or Tcc);

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic amino acid;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl); wherein, in formula (II) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

Preferred compounds of formula (II) include those where X is A^{20}-A^{21}-A^{22}-A^{23}-A^{24}-A^{25}-A^{26} where

A^{20} is Tyr, or any aromatic amino acid;
A^{21} is Tyr, or any aromatic amino acid;
A^{22} is Ser, Thr, N-Me-Ser, or N-Me-Thr;
A^{23} is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, N-Me-Ser, or N-Me-Thr;
A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;
A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5 , independently, is

H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} alkaryl; or a pharmaceutically acceptable salt thereof.

Preferably, said pseudopeptide bond is between amino acid residues A^{29}-A^{30}, A^{34}-A^{35}, and A^{35}-A^{36}.

Preferably, the compound of formula (II) is [D-Trp^{28}, D-Trp^{32}]NPY (27-36), (Des-Asn^{29}[D-Trp^{28}, D-Trp^{32}]NPY(27-36), Des-Asn^{29}[D-Trp^{28}, D-Trp^{32}, Nva^{34}]NPY(27-36), Des-Asn^{29}[Trp^{28}, Trp^{32}, Nva^{34}]NPY(27-36), and [D-Trp^{28}, Ant^{32}, Nva^{34}]NPY(27-36), Des-Asn^{29}[D-Trp^{28}, Ant^{32}, Nva^{34}]NPY(27-36), or Des-Asn^{29}, Arg^{33}[D-Trp^{28}, Ant^{32}, Nva^{34}]NPY(27-36).

In another aspect the invention features a compound having a formula:

$$\begin{array}{l} R_1 \\ \quad \backslash \\ \quad A^1\text{-}A^2\text{-}A^3\text{-}A^4\text{-}A^5\text{-}A^6\text{-}A^7\text{-}A^8\text{-}A^9\text{-}Y\text{-}A^{18}\text{-}A^{19}\text{-}A^{20}\text{-}A^{21}\text{-}A^{22}\text{-}A^{23}\text{-}A^{24} \\ \quad / \\ R_2 \end{array}$$

$$\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36}\text{-}W$$

(III)

wherein a disulfide bond is between A^7 and A^{21} or is absent; wherein each

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

A^1 is Tyr, or any aromatic amino acid;

A^2 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal or Asp;

A^3 is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb, Nle, or N-Me-Leu,

A^4 is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^5 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, or D-Trp;

A^6 is Gly or is the D- or L- isomer selected from the group consisting of Asp, Glu, N-Me-Asp, Ala, or Aoc;

A^7 is Cys, Glu, Asn, Ala, Gln, Gly, or N-Me-Asn;

A^8 is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac_6c, or D-Pal;

A^9 is Gly, N-Me-Gly, Ala, or Trp;

Y is A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17} or is absent, where A^{10} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{11} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{12} is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{13} is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala Thr;

A^{14} is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{15} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{16} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{17} is Met, Leu, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;

A^{18} is, Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{19} is the D- of L- isomer selected from the group consisting of Arg, D-homo-Arg, D-diethyl-homo-Arg, D-Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Cys, Lys, Tyr, or any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala,

A^{23} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a

branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotroptophan, or Tcc);

A^{33} is the D- or L- isomer is selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic amino acid;

W is -OH, -N-R_3R_4, or OR_5 (where R_3, R_4, and R_5, independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl,), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl),

C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl; wherein, in formula (III) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

Preferably, said pseudopeptide bond is between amino acid residues A^{29}-A^{30}, A^{34}-A^{35}, and A^{35}-A^{36}.

Preferably the compound of formula (III) is cyclo(7/21), Des AA^{10-17}[Cys^7, Cys^{21}, D-Trp^{32}]NPY, or cyclo(7/21), Des AA^{10-17}[Glu^7, Lys^{21}, D-Trp^{32}]NPY.

In another aspect, the invention features a compound with pseudopeptide bonds having the formula:

$$\begin{matrix} R_1 \diagdown \\ R_2 - \end{matrix} A^{18}\text{-}A^{19}\text{-}A^{20}\text{-}A^{21}\text{-}A^{22}\text{-}A^{23}\text{-}A^{24}\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}$$

$$A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36} - W \qquad \text{(IV)}$$

wherein each

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

A^{18} is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{19} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Tyr, or any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala,

A^{23} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotroptophan, or Tcc);

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C^6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic acid;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl);

wherein, in formula (IV) each bond can represent either a peptide or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof. In preferred embodiments, the compound contains a pseudopeptide bond between A^{30} and A^{31}; A^{31} and A^{32}; or A^{32} and A^{33}.

In another aspect, the invention features a method of suppressing an NPY mediated physiological response in a tissue other than the heart in a subject comprising administering to said subject a compound having the following formula:

$$\begin{matrix} R_1 \\ R_2 \end{matrix} \diagdown - A^{18}\text{-}A^{19}\text{-}A^{20}\text{-}A^{21}\text{-}A^{22}\text{-}A^{23}\text{-}A^{24}\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}$$

$$A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36} - W$$

wherein each

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl

(e.g., *p*-methylphenyl);

A^{18} is Ala, Asn. Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{19} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Tyr, or any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, or N-Me-Thr;

A^{23} is Ala, Ser, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, D-homo-Arg, D-diethyl-homo-Arg, D-Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring (e.g., Me-Trp);

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-

Tyr, a tethered amino acid with an indole ring (e.g., Me-Trp), Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative (e.g., 2-chlorotroptophan, or Tcc);

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic acid;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl (e.g., methyl), C_6-C_{18} aryl (e.g., phenyl), C_1-C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7-C_{18} aralkyl (e.g., benzyl), or C_7-C_{18} alkaryl (e.g., *p*-methylphenyl); wherein, each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

Preferably, said pseudopeptide bond is between amino acid residues A^{29}-A^{30}, A^{34}-A^{35}, and A^{35}-A^{36}. or a pharmaceutically acceptable salt thereof.

In preferred embodiments, the method suppresses the activity of the NPY (Y-1) receptor or the NPY (Y-2) receptor.

In another aspect, the invention features a method of suppressing a NPY(Y-1) receptor mediated physiological response in the hypothalamus of a subject comprising

administering to said subject the compound of formula (I).

In another aspect, the invention features a method of suppressing the blood pressure of a subject experiencing hypertension which comprises administering to said subject the compound of formula (I).

In another aspect, the invention features a method of suppressing a NPY(Y-3) receptor mediated physiological response in the cardiovascular system of a subject comprising administering to said subject the compound of formula (IV).

In other preferred embodiments, a therapeutically effective amount of a compound of formula (I), (II), (III) or (IV) and a pharmaceutically acceptable carrier substance, e.g., magnesium carbonate or lactose, together form a therapeutic composition capable of suppressing an NPY mediated physiological response. This composition can be in the form a pill, tablet, capsule, liquid, or sustained released tablet for oral administration; or a liquid for nasal administration as drops or spray; or a liquid for intravenous, subcutaneous, parenteral, or intraperitoneal administration.

Another preferred form for administration biodegradable sustained-release composition for intramuscular administration to a subject in need of the composition. Preferably, the composition includes a lipophilic salt and is suitable for administration in the form of an oil emulsion or dispersion to a subject in need of the composition.

In yet another aspect, the invention features methods for suppressing an NPY mediated physiological response in a subject; such methods involve administering one or more of the above mentioned compounds to a subject in a dosage effective to lower blood pressure; to

suppress the appetite; to augment the libido; to stimulate cardiovascular function; on to modulate the circadian rhythm.

In still another aspect, the invention features methods for stimulating an NPY mediated physiological response in a subject; such methods involve administering one or more of the above mentioned compounds to a subject in a dosage effective to increase blood pressure; to increase the appetite; to augment the libido; or to stimulate cardiovascular function.

The symbol A^1, A^2, A^3, and the like; and Tyr, Lys or the like, as found in a peptide sequence herein stands for an amino acid residue, e.g., =N-CH(R)-CO- when it is at the N-terminus, or -NH-CH(R)-CO- when it is at any other position, where R denotes the side chain (or identifying group) of an amino acid or its residue. For example, R is $-CH_2COOH$ for Asp, R is -H for Gly, R is -CH_2OH for Ser, R is $-CH_3$ for Ala and R is $-CH_2CH_2CH_2CH_2NH_2$ for Arg. Also, when the amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated. By pseudopeptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon, i.e., CH_2-NH; or less preferably that of C)-NH is replaced with any of CH_2-S, CH_2-O, CH_2-CH_2, CH_2-CO, or CH_2-CH_2. (A pseudopeptide peptide bond is symbolized herein by or "Ψ".) A detailed discussion of the chemistry of pseudopeptide bonds is given in Coy et al. (1988) *Tetrahedron* 44:835-841.

In other embodiments, the compounds of Formulae (I), (II), (III), or (IV) are cyclic. Preferably, the cyclization is formed by a disulfide or lactam bridge (amide bond). In this disclosure, the disulfide or amide bond which links two residues in a compound of the

invention are formed between the side chain functionalities. That is, between the side-chain carboxyl group of an acidic amino acid residue (e.g., Asp or Glu) and the side chain amino group of a basic amino acid residue (e.g., Lys or Orn), or between the side chain sulfhydryl groups of two Cys. In all formulae set forth herein, the amide or disulfide bond between two residues are not shown. A compound of this invention is also denoted by another format, e.g. cyclo (2/27) Des-AA^{7-24}[Asp^2, D-Ala^6, D-Lys^{27}, D-Trp^{32}] NPY and cyclo(7/21) Des AA^{10-17}[Cys^7, Cys^{21}, D-Trp^{32}]NPY.

Preferred cyclic compounds of the invention are cyclo (2/27) Des AA^{7-24}[Asp^2, D-Ala^6, D-Lys^{27}, D-Trp^{32}] NPY and cyclo(7/21) Des AA^{10-17}[Cys^7, Cys^{21}, D-Trp^{32}]NPY.

In another aspect, the invention features novel dimeric analogs of NPY. The dimer may be formed by either including one compound of Formula I, II, II, or IV and one compound of Formula I, II, III, or IV. In one embodiment, the dimer is formed by utilizing a dicarboxylic acid linker capable of binding to a free amine, either primary or secondary, located within each compound. <u>See</u> R. Vavrek and J. Stewart, Peptides: Structure and Function 381-384 (Pierce Chemical Co. 1983). Examples of suitable dicarboxylic acid linkers are succinic acid, glutamic acid, and phthalic acid. In other embodiments, the dimer is formed by utilizing an amino acid linker capable of binding to a free amine group of one compound and a free carboxylic acid group of the other compound. Preferably, the amino acid linker is a non-α-amino acid. Examples of suitable amino acid linkers are amino-caproic acid and amino-valeric acid. In yet another embodiment, the dimer is formed by disulfide bridge between cysteines located within each compound. <u>See</u> M. Berngtowicz and G. Piatsueda, Peptides: Structure and Function 233-244 (Pierce Chemical Co.

1985); F. Albericio, et al., Peptides 1990 535 (ESCOM 1991).

Preferred dimeric compounds of the invention are Bis(31/31) [Cys^{31}, Trp^{32}, Nva^{34}]NPY(27-36), and Bis(31/31) (Cys^{31}, Trp^{32}, Nva^{34}]NPY(31-36),

As set forth above and for convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art; but for clarity are listed below. All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right. A short line between two amino acid residues indicates a peptide bond.

Abbreviations (common):

Asp = D = Aspartic Acid
Ala = A = Alanine
Arg = R = Arginine
Asn = N = Asparagine
Cys = C = Cysteine
Gly = G = Glycine
Glu = E = Glutamic Acid
Gln = Q = Glutamine
His = H = Histidine
Ile = I = Isoleucine
Leu = L = Leucine
Lys = K = Lysine
Met = M = Methionine
Phe = F = Phenylalanine
Pro = P = Proline
Ser = S = Serine
Thr = T = Threonine

Trp = W = Tryptophan
Tyr = Y = Tyrosine
Val = V = Valine

Abbreviations (uncommon):
Aoc = (8-aminooctanoic acid:
Orn = Ornithine
Nal = 2-napthylalanine
Thi = 2-thienylalanine
Pcp = 4-chlorophenylalanine
Bth = 3-benzothienyalanine
Bip = 4,4'-biphenylalanine
Tic = tetrahydroisoquinoline-3-carboxylic acid
Aib = aminoisobutyric acid
Anb = α-aminonormalbutyric acid
Dip = 2,2-diphenylalanine
Ac_6c = 1-aminocyclohexanecarboxylic acid
D-Pal = β-(3-pyridyl)alanine;
Tcc = tetrahydrocarbolenecarboxylic acid
Nva = norvaline
Ant = anthranilic acid
Hyp = hydroxyproline
Nle = norleucine

The compounds of the invention are useful for reducing, suppressing or mitigating the effects of NPY. For example, the compounds of the invention are especially useful in treating any number of illnesses that involve eating disorders, cardiovascular function, alterations in sexual function, as well as disorders of sleep and circadian rhythms (see, e.g., *Harrison's Principles of Internal Medicine*, McGraw-Hill Inc., New York, 12th ed.). Specific examples of such disorders, include without limitation, obesity, anorexia, hypertension, hypotension, congestive heart failure,

impotence, dyssomnias and rapid time-zone change syndrome. Strategic design of the NPY antagonists, as described herein, allows for the selective antagonism of different classes of NPY receptors, e.g., Y3 cardiac receptors, without adverse interaction with other NPY receptors. The compounds are also useful for stimulating NPY receptor mediated events, e.g., increasing the blood pressure of a subject.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of Preferred Embodiments

The drawings will first be described.

DRAWINGS

Fig. 1 shows the comparison of the effects of D-Trp or D-Trp(CHO) substituted NPY analogs (1.0 μM) on the isoproternol stimulated adenylate cyclase activity of rat hypothalmic membranes. Iso, isoproternol. I., [D-Trp^{32}] Trp^{32}]NPY; II, [D-Trp(CHO)^{32}]NPY; III, [D-Trp^{34}]NPY; IV, [D-Trp(CHO)^{34}]NPY; V, [D-Trp^{36}]NPY; VI, [D-Trp(CHO)^{36}]NPY; a=p,0.01 compared to isoproternol; b, not signifcant compared to isoproternol.

Fig. 2 shows the displacement of ^{125}I-NPY bound to rat hypothalamic membranes by increasing concentrations NPY (•) and [D-Trp^{32}] NPY (□).

Fig. 3 shows the dose-response effects of increasing concentrations of [D-Trp^{32}] NPY (□), NPY alone (•); NPY in the presence of 30 (▲) and 300 (■) nM doses of [D-Trp^{32}] NPY on the isoproterenol stimulated adenylate cyclase activity of rat hypothalamic membranes.

Fig. 4 shows the comparison of the effects of [D-Trp^{32}]NPY (1.0 μM) on the inhibition of isoproterenol stimulated adenylate cyclase activity of rat hypothalamic membranes by NPY (100 nM) and serotonin (100 nM). a = p <

0.01 compared to isoproterenol; b, not significant compared to isoproterenol .

Fig. 5 shows the antagonism of NPY induced feeding in rats by [D-Trp^{32}]NPY.

Fig. 6 shows the effects of 1 μM doses of NPY and its analogs [L-Trp^{32}] NPY, [D-Trp^{32}(CHO)] NPY, [D-Nal^{32}] NPY, [D-Hyp^{32}] NPY, [(3-1-Tyr^{27}), D-Trp^{32}] NPY, and [(3-1-Tyr^{27,36}), D-Trp^{32}] NPY on isoproterenol stimulated adenylate cyclase activity of rat hypothalamic membranes. (iso = isoproterenol); (a = $p < 0.005$ vs. iso.); (n.s. = not significant).

Fig. 7 shows the effects of increasing concentrations of NPY in the absence (O) and presence (•) of Des-AA$^{7-24}$[D-Ala^5, Aoc^6, D-Trp^{32}] NPY (1 μM) on the isoproterenol stimulated cAMP production by SK-N-MC cells. Also shown is the effect of increasing concentrations of Des-AA$^{7-24}$[D-Ala^5, Aoc^6, D-Trp^{32}]NPY (□) on the isoproterenol stimulated cAMP production by SK-N-MC cells.

Fig. 8 shows the effects of increasing concentrations of NPY on the blood pressure of anesthetized rats in the absence (O) and presence (•) of 200 nmol/kg of Des-AA$^{7-24}$[D-Ala^5, Aoc^6, D-Trp^{32}]NPY.

Fig. 9 shows the effects of increasing concentrations of NPY (○) and NPY (18-36) (▵) on the binding of ^{125}I-NPY to SK-N-BE2 cells.

Fig. 10 shows the effects of NPY (○), NPY (18-36) (▵) and NPY in the presence of 1μM dose of NPY (18-36) (•) on forskolin stimulated cAMP production by SK-N-BE2 cells.

Figs. 11A-11C show the analytical RPLC of [Ψ^{30-31}] NPY (18-36) (11A), [Ψ^{32-33}] NPY (18-36) (11B), and [Ψ^{33-34}] NPY (18-36) (11C).

Fig. 12 shows the inhibition of ^{125}I-NPY binding to rat cardiac ventricular membrane by NPY (○), NPY

(18-36) (■), [$\Psi^{30/31}$] NPY (18-36) (△), [$\Psi^{31/32}$] NPY (18-36) (▲), and [$\Psi^{32/33}$] NPY (18-36) (□).

Any number of analogs of the invention can be synthesized and tested in one or more of the assays are described below or by methods which are known in the art. We now describe preferred embodiments of the invention.

STRUCTURE

The sequences of naturally occurring NPY are described *supra*. As is easily observed, there is a high degree of amino acid homology between NPY and PYY.

The analogs of the invention have the general formula recited in the Summary of the Invention *above*. The analogs of the invention are based upon the biologically active full-length molecule (amino acids 1-36) comprising amino acids of NPY and PYY and derivatives thereof; and upon the biologically active subfragments comprising amino acids of NPY and PYY and derivatives thereof.

The analogs of the invention may have one or more modifications to the NPY and PYY sequences (*see above*). For example, the compounds may have one or more of the following modifications which are useful for obtaining selective activity at a NPY receptor: a D-Trp or Aoc or D-Ala in place of one or two or three natural amino acids; or a deletion of several N-terminal amino acids; or the introduction of a pseudopeptide bond instead of a peptide bond between two adjacent amino acids. The analog is capable of acting as a competitive inhibitor of the naturally occurring NPY peptide by binding to the receptor and, by virtue of one of the modifications described *supra* herein, fail to exhibit the biological activity of the naturally occurring peptide. For example, the peptides for which introduction of a pseudopeptide bond between two residues, or the replacement of one or more natural amino acids with a D-

Trp, or the deletion ("des") of the N-terminal residues or internal residues are useful in activity associated NPY activity.

The analogs of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, or pamoic acid, as wells as polymeric acids and slats with inorganic acids such as the hydrohalic acids, e.g., hydrochloric and sulfuric acids.

SYNTHESIS

Peptide Synthesis

The compounds of the present invention , i.e., compounds of formulas (I), (II), (III), (IV), and (V) may be synthesized by any techniques that are known to those skilled in the peptide art. Such techniques are described in, e.g., *Solid Phase Peptide Synthesis*, eds, John M. Stewart and Janis D. Young, Pierce Chemical Company, Rockford, IL, 2nd edition.

The syntheses of the peptides listed in Table 1 and Table 2 were carried out as follows. Peptides were synthesized in an Applied Biosystems model 430A automated instrument, cleaved by hydrogen fluoride, and purified by reversed phase chromatography as described by Balasubramaniam et al. (*Int. J. Pept. Protein Res.* 29:78-83, 1987; *Pept. Res.* 1:32-35, 1988). All synthetic peptides were >98% pure as determined by reverse phase chromatography and had the expected amino acid composition and primary structure. Other analogs can be prepared by making appropriate modifications, within the ability of someone of ordinary skill in this field.

In addition, pseudopeptide bonds may, if desired, may be introduced at various positions, e.g., between amino acid residues 31-32 of NPY(18-36) or between

residues 32-33 of NPY(18-36), or of any peptide as described below. Despite the fact that optically pure Boc-AA-CHO can be obtained in good yields and coupled directly to the α-NH_2 group of the peptide resin by published methods (Sasaki et al., *Peptides* 8:119-121, 1987; Fehrentz et al., *Synthesis* pp.676-678, 1983), this strategy has its limitations because of the possibility of branching at the secondary amine group especially during the synthesis of long peptides with pseudobonds at the C-terminal region. Therefore the utility of several protecting groups, Z, Tos and Z(2-Cl), for capping the secondary amine group in the peptide resin was investigated. Although the reaction of the peptide resin with Z-Cl/Tos-Cl (2 equiv.) & DIEA (4 equiv.) completely blocked the secondary amine, the known lability of Z-during repeated acidolysis to remove Boc group and the apparent resistance of Tos group to HF led us to choose Z(2-Cl) the secondary amine for capping. This is introduced by reacting the peptide resin with Z(2-Cl)-OSU (2 equiv.), HOBT (2 equiv.) and DIEA (4 equiv.) for 10-60 min. The red wine color of ninhydrin with secondary amine turned yellow at the end of capping. This method yielded $[\Psi^{30/31}]$NPY(18-36), $[\Psi^{31/32}]$NPY(18-36) $[\Psi^{32/33}]$NPY(18-36) in greater than 65% yield as judged by analytical HPLC. These peptides not only retained the antagonistic effect, but also exhibited increased affinity (20-220 times) and selectivity for cardiac NPY receptors than NPY(18-36) as discussed below. Integrity of peptides containing pseudobonds were confirmed by mass spectral analysis. Pseudopeptide bond-containing analogs of NPY synthesized by these methods are listed in Table II. Protected amino acid derivatives (Peptide International, Louisville, KY) and peptide synthesis reagents (Applied Biosystems, Foster City, CA) were

obtained commercially and used without further purification.

Examples of the synthesized analogs are:

<u>Formula (I) Compounds</u>

[D-Trp^{32}]NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLI[D-Trp]RQRY-NH$_2$
[D-Nal^{32}]NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLI[D-Nal]RQRY-NH$_2$
[D-Phe^{32}]NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLI[D-Phe]RQRY-NH$_2$
[D-Hyp^{32}]NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLI[D-Hyp]RQRY-NH$_2$
[L-Trp^{32}]NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLI[L-Trp]RQRY-NH$_2$

Des AA$^{7-24}$[D-Trp^{32}]NPY

YPSKPD------------------RHYINLI[D-Trp]RQRY-NH$_2$

Des AA$^{7-24}$[D-Ala^{5}, Aoc^{6},D-Trp^{32}]NPY

YPSK[D-Ala][Aoc]------------------RHYINLI[D-Trp]RQRY-NH$_2$

Des AA$^{7-24}$[Aoc^{6},D-Trp^{32}]NPY

YPSKP[Aoc]------------------RHYINLI[D-Trp]RQRY-NH$_2$

<u>Formula (II) Compounds</u>

[D-Ala^{28},D-Trp^{32}]NPY(27-36)	Y[D-Ala]NLI[D-Trp]RQRY-NH$_2$
Des-Asn^{29}[D-Trp^{28,32}]NPY(27-36)	Y[D-Trp]-LI[D-Trp]RQRY-NH$_2$

<u>Formula (III) Compounds</u>

cyclo(7/21), Des AA$^{10-17}$[Cys^{7}, Cys^{21},D-Trp^{32}]NPY

YPSKPDCPG-------ARYCSALRHYINLI[D-Trp]RQRY-NH$_2$

cyclo(7/21), Des AA$^{10-17}$[Glu^{7}, Lys^{21}, D-Trp^{32}]NPY

YPSKPDEPG--------ARYKSALRHYINLI[D-Trp]RQRY-NH$_2$

<u>Formula (IV) Compounds</u>

[$^{30/31}$]NPY (18-36)	------------------ARYYSALRHYINL ITRQRY-NH$_2$
[$^{31/32}$]NPY (18-36)	------------------ARYYSALRHYINLI TRQRY-NH$_2$
[$^{32/33}$]NPY (18-36)	------------------ARYYSALRHYINLIT RQRY-NH$_2$

Other analogs of the invention can be prepared as above and tested for their biological activity effectiveness as antagonists or agonists using the methods described below and those commonly known in the art.

FUNCTIONAL ASSAYS

Animals, Cell Lines and Cultures, and Reagents

Any suitable in vivo or in vitro system may be utilized to assay and test the effectiveness of the compounds of the invention. Such assays may employ in vivo methods for evaluating physiological responses, e.g., blood pressure, renovascular function, feeding behavior, or circadian rhythm, or in vivo biochemical systems evaluating receptor binding in a suitable cell line, e.g., SK-N-MC (ATCC#HBT 10) or SK-N-BE(2) (Barnes et al. *In Vitro* 17: 619-631, 1981); or in isolated cells, e.g., cells isolated from the spleen, kidney, heart or brain. A number of in vivo and in vitro biochemical systems known to those skilled in the art are available for testing antagonists to NPY receptors, e.g. the Y-1, Y-2, and Y-3 receptor categories. Described below are assay methods which can be utilized with cell lines such as SK-N-MC and SK-N-BE2 or isolated cardiac membranes which possess the high-affinity NPY receptor sites Y-1, Y-2, and Y-3, respectively. Other systems are also known for evaluating NPY antagonists to the Y-1 receptor, e.g. VSM cells (Sheikh et al., *Am. J. Physiol.* 260: G250-G257, 1991) and HEL cells (Motulsky et al. *Amer. J. Physiol.* 255: E880-E885, 1988); Y-2 receptor, e.g., kidney (Sheikh et al., Am. J. Physiol 26:F978-F984), spleen (Lunberg et al., Eur. J. Pharmal. 145:21-29, 1988), dorsal root ganglion (Bleakman et al., Br. J. Pharmal. 103:1781-1789, 1991) and hippocampal cells (Sheikh et al., J. Biol. Chem. 265:8304-8310, 1990); and Y-3 receptors, e.g., in cardiac ventricular membranes (Balasubramaniam et al.,

Peptides 11: 545-550, 1990), chromaffin cells, rat gastric mucosa (Michel, M.C., *Trends in Pharmol. Sci.* 12: 389-394, 1991) and brain stem.

In Vitro Biochemical Assays

The ability of the compounds of the invention to act as antagonists of NPY can be demonstrated by any number of methods known in the art. For example, the compounds can be shown to compete with iodinated neuropeptide Y for receptors using the methods described by Lundberg et al. (*Eur. J. Pharmol.* 145: 21-29, 1988); Gordon et al. (*J. Neurochemistry* 55:506-513, 1990); Walker et al. (*Mol. Pharmacol.* 34:779-792, 1988); Balasubramaniam et al. (*Peptides* 10:1283-1286, 1989), and others.

In one working example demonstrating antagonists to Y-1 receptors, rat hypothalamus was isolated and the membranes were prepared for binding and adenylate cyclase studies according to standard methods (Unden et al. 1984. *Eur. J. Biochem* 145: 525-530; Westlind-Danielsson et al. 1987. *Neurosci. Lett.* 74: 237-242). Displacement studies were performed in a total volume of 0.25 ml 20 mM HEPES buffer, pH 7.4, containing 1% bovine serum albumin, 0.1% bacitracin, 300 μm PMSF and 5 KIU/ml aprotinin. In a standard assay, 100 μg of membrane/tube was incubated in a shaking water bath at 24° C for 45 min with [^{125}I-Tyr^{1}]-NPY (20,000 CPM) as described by Balasubramaniam et al (*Peptides* 11: 545-550, 1990) in the presence of increasing concentrations of NPY (10^{-11}-10^{-5} M). At the end of incubation, 1.0 ml of iced cold buffer was added, centrifuged at 10,000 X *g* for 10 min, and the supernatant removed by aspiration. The tube containing the pellet was counted for bound radioactivity in a micromedic gamma-counter.

An example of assaying adenylate cyclase activity of hypothalamic and cerebral cortex membranes is now

described. Adenylate cyclase activity of the hypothalamic and cerebral cortex membranes was determined by incubating 50 μg of membranes in a total volume of 0.20 ml Tris-HCL 30 mM pH 7.4 buffer containing 150 mM NaCl, 8.25 mM $MgCl_2$, 0.75 mM EGTA, 1.5 theophylline, 20 μg/ml aprotinin, 100 μg/ml bacitracin, 1 mg/ml bovine serum albumin, 1 mM ATP, 20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase, 10 μM isopreternol, 10 μM GTP, and various concentrations of peptides (0-10 μM). After incubating the mixture at 35° C for 15 min in a shaking water bath, the reaction was arrested by the addition of 100 μM EDTA and boiling for 3 min. cAMP was extracted and quantitated by radioimmunoassay. All the points in the binding and adenylate cyclase are the means of at least three parallel experiments performed in duplicate.

In one working example demonstrating antagonists to Y-3 receptors, rat cardiac ventricular membranes and iodination of NPY were prepared according to the method described by Balasubramaniam et al. (*Peptides* 11: 545-550, 1990). Displacement studies were performed in a total volume of 0.25 ml of 20 mM HEPES assay buffer, pH 7.6, containing 2% bovine serum albumin, 100 μM phenylmethylsulfonyl fluoride, 4 μg/ml leupeptin, 4 μg/ml chymostatin, 5 kallikrein-inactivating units/ml aprotinin, and 0.1% bacitracin. In a standard assay, 200 μg of membrane protein/tube were incubated for 2 h at 18°C in a shaking water bath with ^{125}I-NPY (40 pM) and increasing concentrations of peptides. At the end of incubation, tubes were vortexed and 150μl aliquots transferred into polypropylene tubes containing 250 μl of ice-cold assay buffer. Unbound ^{125}I-NPY was separated by centrifugation at 10,000 x g for 10 min followed by aspiration of the supernatant. The tubes containing the pellet were counted for bound radioactivity in a

Micromedic γ counter. The IC_{50} values were used to calculate the equilibrium dissociation constant, K_i for NPY and NPY antagonists using the equation $K_i = IC_{50}/(1 + F/K_d)$, where F and K_i denote the concentration and the dissociation constant of ^{125}I-NPY.

Adenylate cyclase activity was measured by Rosselin et al. (*Biochim. Biophys. Acta* 304:541-551, 1977). Each experiment was carried out in a total volume of 200 μl solution containing 30 mM Tris-HCl, pH 7.4, 150 mM NaCl, 8.25 mM $MgCl_2$ 0.75 mM EGTA, 1.5 mM theophylline, 20 μg/ml aprotinin, 100 μg/ml bacitracin, 1 mg/ml BSA, 1 mM ATP, 20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase, 10 μM isoproterenol, 10 μM GTP, and various concentrations of peptides (0-10 μM). The reaction was initiated by the addition of 50 μg (50 μl) of membrane protein. After incubation at 35°C for 10 min. in a shaking water bath, the reaction was terminated by the addition of 100 μM EDTA and boiling for 3 min. cAMP was extracted and quantitated by radioimmunoassay using a kit obtained from New England Nuclear, Boston, MA.

<u>In Vivo Assays</u>

Any suitable <u>in vivo</u> model system can be used to evaluate the antagonistic properties of the compounds of the invention. Such models, without limitation, include those used to evaluate feeding and memory behavior (Flood et al., *Peptides* 10:963-966), and vasoconstriction and hypertension (Balasubramaniam et al. *Biochim et Biophys Acta* 997: 176-188, 1989).

Thus, in one working example, feeding studies were performed using Sprague Dawley rats (350-450 g) with paraventricular hypothalamic cannulae to investigate effects of NPY analogs (Chance et al. 1989. *Peptides* 10: 1283-1286). Antagonism of NPY induced feeding in rats was by [D-Trp^{32}]NPY. Groups of rats received intrahypothalamic injections (1 μl) of artificial CSF or

10 μg of [D-Trp^{32}]NPY. Fifteen minutes later CSF-treated rats were injected with CSF (n = 6), 1 μg of NPY (n = 6) or 10 μg of [D-Trp^{32}]NPY (n = 7), while the [D-Trp^{32}]NPY-treated rats were injected with 1 μg of NPY (n = 8). Rats were provided with a known quantity of rat chow, and after 1 hr the food consumed was determined and corrected for spillage a = $p < 0.01$ vs. CSF; b, not significant vs. CSF; c = $p < 0.01$ vs. NPY; d = $p < 0.05$ vs. NPY.

In another working example blood pressure studies were performed to evaluate the antagonistic properties of Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY. The method is as follows, after surgical preparation, three doses of NPY (0.1, 1.0 and 10 nmol/kg) were administered by intravenous push to 7 rats in a randomized order. Each dose was separated by a 20 minute washout period. After obtaining baseline systolic blood pressure (SBP) values, the rats received either 200 nmol/kg of Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY (n=5) or 0.9% saline (n=2) prior to each NPY dose. Change in SBP from basal state to maximum SBP observed following NPY was compared between baseline and Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY treatments. The duration of SBP effect of Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY was determined in 3 animals by administering 1.0 nmol/kg of NPY every 15 minutes for 75 minutes following a single 200 nmol/kg dose of Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY.

RESULTS

We first synthesized a series of full length analogs of NPY substituting either D-Trp or D-Trp(CHO) in the C-terminal receptor binding region at positions 32, 34 and 36. We tested for agonist activity on isoproterenol-stimulated hypothalamic adenylate cyclase activity. Fig. 1 shows that at 1.0 μM, NPY, [D-Trp^{34}]NPY, [D-Trp^{36}]NPY, and the corresponding formulated D-Trp

analogs inhibited isoproterenol-stimulated hypothalamic adenylate cyclase activity significantly. [D-Trp^{32}]NPY and its formulated derivative, however, did not exhibit significant inhibitory effect on adenylate cyclase activity at this concentration. In the binding experiments shown in Fig. 2, NPY and [D-Trp^{32}]NPY inhibited ^{125}I-NPY bound to rat hypothalamic membranes in a dose-dependent manner with IC_{50} values of 0.63 nM and 3.0 nM, respectively. It is this high receptor activity and the complete loss of intrinsic activity that suggests that [D-Trp^{32}]NPY may be an antagonist of NPY in rat hypothalamus.

The complete loss of intrinsic activity, while retaining high binding potency suggested that [D-Trp^{32}]NPY may be an antagonist of NPY in hypothalamus. In order to further substantiate this observation, we investigated the inhibitory effect of NPY on rat hypothalamic membrane adenylate cyclase activity both in the absence and presence of [D-Trp^{32}]NPY. Fig. 3 shows that NPY inhibited isoproterenol stimulated hypothalamic membrane adenylate cyclase activity dose-dependently with an IC_{50} value 0.18 nM. [D-Trp^{32}]NPY did not exhibit any inhibitory effect on adenylate cyclase activity. Further, Fig. 3 shows that the presence of 30 and 300 nM [D-Trp^{32}]NPY shifted the inhibitory dose-response curve of NPY on hypothalamic adenylate cyclase activity to the right increasing that IC_{50} value to 4.0 nM (K_B = 1.41 nM) and 540. nM (K_B = 1.36 nM), respectively.

To assess the specificity of [D-Trp^{32}]NPY, we investigated its effect on the inhibitory hypothalamic adenylate cyclase activity of serotonin. Fig. 4 shows that the presence of serotonin (100 nM) significantly ($p < 0.01$; by repeated measures ANOVA) inhibited the isoproterenol stimulate adenylate cyclase activity both in the absence and presence of [D-Trp^{32}]NPY (1 μM). The

antagonism at [D-Trp^{32}]NPY, therefore, was specific to the NPY receptor since the analog exhibited no effect on the inhibitory hypothalamic AC activity of serotonin and, thus, did not act as a global antagonist.

Since hypothalamic NPY has been shown to elicit a feeding response, we also investigated the effect of [D-Trp^{32}]NPY on NPY induced feeding in freely moving rats. Fig. 5 shows that intrahypothalamic injection of NPY (1 μg) significantly ($p < 0.01$) stimulated the cumulative food intake as compared to vehicle (artificial cerebrospinal fluid) treatment over 1 hr. On the other hand, [D-Trp^{32}]NPY (1 μg) did not stimulate feeding significantly over this period, nor did it attenuate NPY (1 μg) - induced feeding at this concentration. 10 μg of [D-Trp^{32}]NPY also did not exhibit significant effect on feeding, and at this dose significantly ($p < 0.05$) attenuated the 1 hr. cumulative food intake induced by 1 μg of NPY. All of these observations suggest that D-Trp^{32} is a specific and competitive antagonist at NPY in rat hypothalamus in both <u>in vitro</u> and <u>in vivo</u> models.

In order to improve the potency and/or selectivity, several analogs were synthesized substituting the residue at 32 with various amino acids, e.g., D-Nal, D-Phe, D-Hyp, or L-Trp (Fig. 6). However, these analogs exhibited agonistic activity which suggests there are strict structural requirements to induce antagonistic properties to NPY. Although it is generally believed that the NPY effects on blood pressure and feeding are mediated by the Y-1 receptor subtype, it is possible that NPY analogs which elicit pressor effects have no orexigenic effects. Thus, [D-Trp32]NPY is useful not only to elucidate the receptor subtypes mediating NPY effects on hypothalamus, but also to determine whether feeding and pressor effects are mediated by the Y-1 receptors.

Next, the relative binding affinities of various compounds having formula (I) were investigated using SK-N-MC (Y-1) and SK-N-BE2(Y-2) shown in Table I. These studies led to the development of two truncated peptide analogs, Des-AA^{7-24}[Aoc^6, D-Trp^{32}]NPY and Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY, which did not inhibit the cAMP production by SK-N-MC cells (see Table I). However, Des-AA^{7-24}[Aoc^6, D-Trp^{32}]NPY exhibited poor affinity to Y-1 receptors (Table I), and therefore, failed to antagonize the inhibitory effects of NPY on SK-N-MC cAMP production. On the other hand, Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY, surprisingly exhibited moderate affinity (Table I), and its presence (1.0 μM) shifted the inhibitory dose-response curve of NPY on SK-N-MC cAMP production parallel to the right (Fig. 7). These observations confirm that Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY is a competitive antagonist of NPY in Y-1 receptors.

To investigate whether these compounds retained antagonistic activity within an <u>in vivo</u> model, we tested the effects on NPY-induced anorectic rats. Fig. 8 shows that NPY doses of 0.1, 1.0 and 10.0 nmol/kg, during baseline, increased systolic blood pressure (SBP) by 8 ± 7, 26 ± 6 and 37 ± 7 mmHg respectively. Following administration of Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY, NPY doses of 0.1, 1.0 and 10.0 nmol/kg increased SBP by 4 ± 5, 9 ± 5 and 29 ± 17 mmHg respectively. The change in SBP during Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY was significantly different than baseline values ($p = 0.0002$ at the 1.0 nmol/kg NPY doses, but not at the 0.1 or 10 nmol/kg doses. Changes in SBP in control rats receiving saline were not significantly different than baseline values at all NPY doses. The duration of effect of the antagonist ranged between 30-75 minutes. This result demonstrates that Des-AA^{7-24}[D-Ala^5, Aoc^6, D-Trp^{32}]NPY is effective in attenuating NPY induced vasoconstriction <u>in</u>

vivo. Its ability to only affect SBP at the middle NPY dose and the finding that Des-AA$^{7-24}$[D-Ala^{5}, Aoc^{6}, D-Trp^{32}]NPY can inhibit the binding of ^{125}I-NPY to SK-N-MC cells, suggests that Des-AA$^{7-24}$[D-Ala^{5}, Aoc^{6}, D-Trp^{32}]NPY competitively antagonizes NPY induced hypertension.

In addition, further truncation and deletion of Des-AA$^{7-24}$[D-Ala^{5}, Aoc^{6}, D-Trp^{32}]NPY resulted in the development of three analogs (Table I). Although these analogs did not bind to Y-2 receptors, both [D-Ala^{28}], D-Trp^{32}]NPY(27-36) and [Bip^{27}, D-Ala^{28}, D-Trp^{32}]NPY(27-36) also exhibited poor affinity to Y-1 receptor. However, Des-Asn^{29}[D-Trp^{28,32}]NPY(27-36) bound with moderate potency to Y-1 receptors, and also did not exhibit any intrinsic activity on isoproterenol stimulated cAMP production by SK-N-MC cells. These observations suggest that Des-Asn^{29}[D-Trp^{28,32}]NPY(27-36) or its analogs will prove useful for the development low molecular weight selective antagonist compounds for Y-1 receptors.

TABLE I

Peptides	IC_{50} (nM) for the inhibition of ^{125}I-NPY binding to SK-N-MC (Y-1)	SK-N-BE2 (Y-2)
NPY	1.3	0.1
[D-Trp^{32}]NPY	1000	0.63
Des-AA$^{7-24}$[Aoc^{6},D-Trp^{32}]NPY	3900	10.0
Des-AA$^{7-24}$[D-Ala^{5}, Aoc^{6},D-Trp^{32}]NPY	100	1.0
[D-Ala^{28}, D-Trp^{32}]NPY(27-36)	630	N.I.
[Bip^{27}, D-Ala^{28}, D-Trp^{32}]NPY(27-36)	1300	N.I.
Des-Asn^{29} [D-Trp^{28,32}]NPY(27-36)	170	N.I.

N.I.: no inhibition even at 10,000 nM

The analogs of the invention may also be assayed and tested for NPY receptor Y-2 activity using the methods described *supra*. Thus, a compound, e.g., [D-Trp^{32}]NPY, can be assayed for antagonism using any Y-2 receptor bearing cell, e.g., the SK-N-BE2 cell line, or such cells found in the spleen, kidney, hippocampus or dorsal root ganglion.

Towards developing selective agonists and antagonists of Y-2 receptors, we tested a number of compounds using SK-N-BE2 cell lines. These studies demonstrated that NPY(18-36), previously shown to be an antagonist of NPY in rat cardiac membranes bearing Y-3 receptors, antagonizes the inhibitory effect on the cAMP production of SK-N-BE2 cells bearing Y-2 receptor subtypes as shown in Figures 9 and 10.

NPY RECEPTOR (Y-3 SUBTYPE)

Next, we investigated the effect of introducing a pseudopeptide bond to NPY*18-36). Table II shows the results for the increased affinity and selectivity of pseudopeptide analogs of NPY(18-36) for Y-3 receptors. The introduction of pseudobonds ($-CH_2NH-$) at positions 31-32 or 32-33 of NPY(18-36) was found to substantially increase Y-3 receptor affinity (see Table 2). Subsequent experiments revealed that all these analogs retain their antagonistic properties. Furthermore, [$\psi^{30/31}$]NPY(18-36) and [$\psi^{31/32}$]NPY(18-36) and [$\psi^{32/33}$]NPY(18-36) analogs exhibit lower affinity to Y-1 and Y-2 subtypes than NPY(18-36) (Table II). Thus, introduction of pseudobonds at 32-33 and 31-32 also increases their selectivity for Y-3 receptors.

TABLE II

PEPTIDES	IC_{50} (nM) for the inhibition of ^{125}I-NPY binding to:		
	Y-3 (CARDIAC)	Y-2 (SK-N-BE2)	Y-1 (SK-N-MC)
NPY	0.20	0.1	1.3
NPY(18-36)	126	3.00	251
$[\Psi^{32-33}]$NPY(18-36)	0.56	158	1585
$[\Psi^{31-32}]$NPY(18-36)	1.00	562	1995
$[\Psi^{30-31}]$NPY(18-36)	6.00	281	N.D.

Ψ, $-CH_2NH-$; N.D., not determined.

EXAMPLES

This invention is further illustrated by the following nonlimiting examples.

EXAMPLE 1

Synthesis of [D-Ala^5, Aoc^6, D-Trp^{32}]NPY

Peptide Synthesis -- MBHA resin (0.45 mM NH_2 group) was placed in a reaction vessel of the Applied Bioscience (ABI) 430A automated instrument and amino acid derivatives were coupled automatically using the standard program provided by the manufacturer modified to incorporate a double coupling procedure. All amino acids were coupled using 2.2 equivalents of preformed symmetrical anhydrides. Arg, Asn and Gln, however, were coupled as preformed 1-HOBT esters (4.4 equal.) to avoid deamidation or lactam formation. At the end of the

synthesis N-α-Boc-group was removed and peptide resin (~1g) was treated with HF as described below.

In the reaction vessel 1.0 g peptide resin, 0.8 g p-cresol, 0.2g thiocresol, 0.8 ml $(CH_3)_2$ and 5 ml HF were stirred for 40 min of reaction and an additional 60 min. of HF evacuation. During these procedures temperature of reaction vessel was kept between 0°C - ⁻4°C. Then the peptide resin was transferred into a fitted filter funnel in Et_2O and washed with excess of Et_2O. Free peptide was extracted with 30% HOAc (2x15ml). Peptide solution was diluted to 10% HOAc (60ml H_2O) and lyophilized. 390 mg crude peptide was obtained from this procedure.

EXAMPLE 2

Synthesis of [D-Trp^{32}] NPY

Peptide synthesis was performed as described above. Cleavage by HF was as follows: in a reaction vessel 1.0g peptide resin, 0.8 ml $(CH_3)C_2S$, 0.8g p-cresol, 0.2g p-thiocreosl and 5ml HF were stirred for 40 min of reaction in temperature between 0°C - ⁻4°C. After that HF was evacuated in 60. Temperature was still kept below 0°C. The peptide resin was transferred into fitted filter funnel and washed with excess of ET_2O. The peptide resin extracted with 30ml 30% HOAc. Peptide solution was diluted to 10% HOAc with 60ml H_2O and protein lyophilized. Total weight of crude peptide: 190mg.

EXAMPLE 3

Synthesis of Cyclo(7/21), Des-AA$^{10-17}$[Cys^{7,21},D-Trp^{32}] NPY

Peptide synthesis was as described above using an Automated ABI 430A synthesizer. The free peptide was obtained by treating the protected peptide resin (1.0g) with HF (10 ml) containing dimethyl sulfide (0.8 ml), p-cresol (0.2g) for 1 h at -2 to -4 C. The residue was

transferred to a fitted filter funnel with diethyl ether, washed repeatedly with diethyl ether, and the peptide extracted with 10% HOAC(2X 15 ml) and lyophilized. The crude peptide (100mg) thus obtained was dissolved in 6M guanidine HCL (6 ml) diluted with 500 ml of distilled water and the pH adjusted to 8 with ammonia. A solution of potassium ferricyanide (1% w/v) was gradually added with constant stirring until a yellow color persisted. After stirring for an additional 30 min., the pH of the solution was adjusted to 5 with acetic acid and the solution stirred with an anion exchange resin (AG-3, Cl- form, 10g wet weight) for 30 min, passed through a 0.45 microns filter, and pumped into a semipreparative column (250X10 mm), washed with 0.1%TFA-H_2O until a flat base line was obtained. The column containing the peptide was then subjected to gradient elution as described for NPY, and the purified peptide was characterized by amino acid and mass spectral analysis.

EXAMPLE 4

Synthesis of Cyclo(7/21), Des-AA$^{10-17}$[Glu^7, Lys^{21}, D-Trp^{32}]NPY

The synthesis of this peptide was accomplished using the general strategy described for NPY except for the following: After coupling BocGlu(OFM) at position 7, the side chain protecting groups, ϵ-Fmoc group at Lys^{21} and the γORm of Glu^7 were removed by removing the peptide resin with 20% piperidine-DMF. After repeated washings with DMF, the ϵ-NH_2 group of Lys^{21} was coupled to γ-COOH of Glu^7 by stirring the peptide resin with BOP-HOBT-DIPEA (1:1:3) in DMF (20 ml) overnight, and if cyclization is not complete as judged by the standard ninhydrin test the procedure was repeated until complete cyclization has occurred. The synthesis was then continued in the

automated mode, and the free peptide was obtained by the standard HF method described in Example 3.

Example 5

Synthesis of [ψ32/33]NPY (18-36)

Standard techniques, as described above, were employed for the solid phase synthesis of the carboxy terminal portion of cardiac receptor antagonist, NPY [ψ32/33]NPY (18-36), up to the point at which introduction of the pseudopeptide bond was desired. The pseudopeptide bond was then introduced in the analog according to the method of Sasaki et al.(*Peptides* 8:119-121, 1986) , with Boc as the protecting group for the primary amine.

The resulting N-α-Boc-peptide-resin with the pseudopeptide bond (0.25 mmol) was swollen in DMF (10 ml) for 10 min in a two-necked R.B. flask fitted with a drying tube. This was followed by the addition of diisopropylethyl amine (1.0 mmol), HOBt (0.5 mmol) and Z(2-Cl)OSU (0.5 mmol). HOBt enhances the coupling of Z(2-Cl) to the secondary amino group of the pseudopeptide bond. The reaction mixture was stirred at room temperature until the Kaiser's ninhydrin test gave a yellow color indicating that the secondary amine had been blocked. The peptide resin was returned to the reaction vessel of the automated peptide synthesizer and the rest of the sequence was assembled automatically. The free peptide was obtained by the standard cleavage conditions and purified by reverse phase chromatography.

USE

Because NPY is a potent vasoconstrictor and or orexigenic agent, as well as an inhibitor of libido and effector of circulation rhythm, it is likely that the administration of one or more compounds of the invention may suppress or inhibit the deleterious effects of NPY.

Therefore, the NPY antagonists of the invention are suitable for the treatment of any number of diseases related to cardiovascular function (e.g., congestive heart failure or hypertension), obesity, anorexia, blood pressure, asthma, pulmonary hypertension, renal hypertension, memory retention, sexual dysfunction (e.g. impotence), and disorders involving sleep and circadian rhythms. For example, the compounds of formula (I), (II), (III) are useful for treating for controlling feeding disorders and blood pressure; the compounds of formula (IV) are useful for treating any number of heart ailments, e.g., chronic heart failure, as well as promoting recovery from ischemia since the compounds are expected to enhance myocardium contraction; and the compounds of formula (IV) are useful for controlling NPY actions mediated by Y-2 receptor subtypes, e.g., for controlling the effects of NPY on renal blood flow, glomerular filtration rate, natriuresis and renin secretion.

Thus to treat the above disorders, the appropriate NPY antagonist is administered as a therapeutic preparation (as described below) in accordance with the condition to be treated. In the practice of the method of the present invention, an effective amount of an NPY antagonist, e.g., Ψ^{30-31}NPY(18-36), is administered via any of the usual and acceptable methods known in the art, either singly or in combination with another compound or compounds of the present invention. These compounds or compositions can thus be administered orally, sublingually, parenterally (e.g., intramuscularly, intravenously, subcutaneously, or intradermally) or by inhalation, and in the form or either solid, liquid or gaseous dosage, including tablets and suspensions. The administration can be conducted in a single unit dosage

form with continuous therapy or in a single dose therapy ad libitum.

The dose of the compound of the present invention for treating the above-mentioned disorders varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated, and ultimately will be decided by the attending physician or veterinarian. Such amount of the active compound as determined by the attending physician or veterinarian is referred to herein as a "therapeutically effective amount". Thus, a typical administration is oral administration or parenteral administration. The daily dose in the case of oral administration is typically in the range of 0.1 to 100 mg/kg body weight, and the daily dose in the case of parenteral administration is typically in the range of 0.001 to 50 mg/kg body weight.

To be effective for the prevention or treatment of the above-mentioned disorders it is important that the therapeutic agents be relatively non-toxic, non-antigenic and non-irritating at the levels in actual use.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Other embodiments are within the following claims.

CLAIMS

1. A compound having the formula:

$$\begin{array}{l} R_1 \diagdown \\ R_2 - A^1\text{-}A^2\text{-}A^3\text{-}A^4\text{-}A^5\text{-}A^6\text{-}Y\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}A^{33}\text{-} \\ \qquad\qquad A^{34}\text{-}A^{35}\text{-}A^{36} - W \end{array} \qquad (I)$$

wherein

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^1 is Tyr, or any aromatic amino acid;

A^2 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal or Asp;

A^3 is Ser, Thr, N-Me-Ser, N-Me-Thr,Ile, Val, Aib, Anb, Nle, or N-Me-Leu;

A^4 is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^5 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, or D-Trp;

A^6 is Gly or is the D- or L- isomer selected from the group consisting of Asp, Glu, N-Me-Asp, Ala, or Aoc;

Y is A^7-A^8-A^9-A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17}-A^{18}-A^{19}-A^{20}-A^{21}-A^{22}-A^{23}-A^{24} or is absent, where

A^7 is Asn, Ala, Gln, Gly, or N-Me-Asn;

A^8 is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac_6c, or D-Pal;

A^9 is Gly, N-Me-Gly, Ala, or Trp;

A^{10} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{11} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{12} is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{13} is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, or Thr;

A^{14} is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{15} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{16} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{17} is Met, Leu, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;

A^{18} is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{19} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Tyr, any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{23} is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, N-Me-Ser, or N-Me-Thr;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer of selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic amino acid;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl; wherein, in formula (I) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein Y (A^7-A^{24}) is absent.

3. The compound of claim 2, wherein said compound has the formula Des AA^{7-24}, Aoc^6 D-Trp^{32}] NPY.

4. The compound of claim 2, wherein said compound has the formula Des AA $^{7-24}$ [D-Ala^5, Aoc^6, D-Trp^{32}] NPY.

5. A compound having the formula:

$$R_2 - \overset{R_1 \diagdown}{X}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36}\text{-}\ W \qquad (II)$$

wherein X is a chain of 0-7 amino acids, inclusive, the N-terminal one of which is bonded to each R_1 and R_2;

wherein each R_1 and R_2, independently, is each H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, or N-Me-Asn, or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu, or is deleted;

A^{31} is Ile, Cys, D-Ala, Leu, Val, Aib, Anb, or N-Me-Ile, or is deleted;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic amino acid;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} alkaryl or C_7-C_{18} alkaryl; wherein, in formula (II) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

6. The compound of claim 5, where X is A^{20}-A^{21}-A^{22}-A^{23}-A^{24}-A^{25}-A^{26} wherein

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Tyr, or any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr;

A^{23} is Ala, Ser, Thr, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, N-Me-Ser, or N-Me-Thr;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

W is -OH, -N-R_3R_4, or OR_5 (where each R_3, R_4, and R_5, independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

or a pharmaceutically acceptable salt thereof.

7. The compound of claim 5 having the formula [D-Ala^{28}, D-Trp^{32}]NPY (27-36).

8. The compound of claim 5, having the formula Des-Asn^{29} [D-Trp^{28,32}]NPY(27-36).

9. A compound having the formula:

$$\begin{array}{l} R_1 \\ \quad\backslash \\ \quad A^1\text{-}A^2\text{-}A^3\text{-}A^4\text{-}A^5\text{-}A^6\text{-}A^7\text{-}A^8\text{-}A^9\text{- } Y \text{ -}A^{18}\text{-}A^{19}\text{-}A^{20}\text{-}A^{21}\text{-}A^{22}\text{-}A^{23}\text{-}A^{24} \\ \;/ \\ R_2 \end{array}$$

$$\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36}\text{-}W \quad \text{(III)}$$

wherein a disulfide bond is between A^7 and A^{21} or is absent; wherein

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^1 is Tyr, or any aromatic amino acid;

A^2 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal or Asp;

A^3 is Ser, Thr, N-Me-Ser, N-Me-Thr, Ile, Val, Aib, Anb, Nle, or N-Me-Leu

A^4 is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^5 is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, or D-Trp;

A^6 is Gly or is the D- or L- isomer selected from the group consisting of Asp, Glu, N-Me-Asp, Ala, or Aoc;

A^7 is Cys, Glu, Asn, Ala, Gln, Gly, or N-Me-Asn;

A^8 is Pro, Ser, Thr, Hyp, D-Ala, N-Me-Ala, Ac_6c, or D-Pal;

A^9 is Gly, N-Me-Gly, Ala, or Trp;

Y is A^{10}-A^{11}-A^{12}-A^{13}-A^{14}-A^{15}-A^{16}-A^{17} or is absent, where

A^{10} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{11} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{12} is Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{13} is Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, or Thr;

A^{14} is Ala, Pro, Hyp, D-Ala, N-Me-Ala, Ac_6c, D-Pal, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{15} is Glu, Asp, N-Me-Glu, Ala, or Nva;

A^{16} is Asp, Glu, N-Me-Asp, Ala, or Anb;

A^{17} is Met, Leu, Ile, Val, Aib, Anb, Nle, or N-Me-Leu;

A^{18} is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{19} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Cys, Lys, Tyr, or any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{23} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn, or deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or

straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic amino acid;

W is -OH, -N-R_3R_4, or OR_5 (where R_3, R_4, and R_5 , independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl; wherein, in formula (III) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 3 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

10. The compound of claim 9, having the formula cyclo(7/21), Des AA^{10-17}[Cys^7, Cys^{21}, D-Trp^{32}]NPY.

11. The compound of claim 9, having the formula cyclo(7/21), Des AA^{10-17}[Glu^7, Lys^{21}, D-Trp^{32}]NPY.

12. A compound with pseudopeptide bonds having the formula:

$$\begin{matrix} R_1 \\ R_2 \end{matrix} \!\!> A^{18}\text{-}A^{19}\text{-}A^{20}\text{-}A^{21}\text{-}A^{22}\text{-}A^{23}\text{-}A^{24}\text{-}A^{25}\text{-}A^{26}\text{-}A^{27}\text{-}A^{28}\text{-}A^{29}\text{-}A^{30}\text{-}A^{31}\text{-}$$

$$A^{32}\text{-}A^{33}\text{-}A^{34}\text{-}A^{35}\text{-}A^{36} - W \qquad \text{(IV)}$$

wherein

each R_1 and R_2, independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl;

A^{18} is Ala, Asn, Gln, Gly, N-Me-Asn, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{19} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{20} is Tyr, or any aromatic amino acid;

A^{21} is Tyr, or any aromatic amino acid;

A^{22} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala

A^{23} is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Nal, Thi, Phe, Bth, Pcp, or N-Me-Ala;

A^{24} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{25} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{26} is the D- or L- isomer selected from the group consisting of His, Thr, 3-Me-His, β-pyrazolylalanine, N-Me-His, Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{27} is the D- or L- isomer selected from the group consisting of any aromatic amino acid, Lys, or a tethered amino acid with an indole ring;

A^{28} is Aib or is the D- or L- isomer selected from the group consisting of Ile, Leu, Val, Anb, Trp, N-Me-Ile, or is deleted;

A^{29} is Asn, Ala, Gln, Gly, N-Me-Asn, or is deleted;

A^{30} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{31} is Ile, Cys, Leu, Val, Aib, Anb, or N-Me-Ile;

A^{32} is the D- or L- isomer selected from the group consisting of any aromatic amino acid except L-Tyr, a tethered amino acid with an indole ring, Ant, Ser, N-Me-Ser, Thr, N-Me-Thr, Ala, N-Me-Ala, D-Hyp, or any Trp derivative;

A^{33} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), Orn, or is deleted;

A^{34} is Gln, Asn, N-Me-Gln, Nle, Nva, Ala, or Gly;

A^{35} is the D- or L- isomer selected from the group consisting of Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys-ϵ-NH-R (where R is H, a branched or straight chain C_1-C_{10} alkyl group, or a C_6-C_{18} aryl group), or Orn;

A^{36} is Tyr, or any aromatic acid;

W is -OH, -N-R_3R_4, or OR_5 (where R_3, R_4, and R_5, independently, is H, C_1-C_{12} alkyl, C_6-C_{18} aryl, C_1-C_{12} acyl, C_7-C_{18} aralkyl, or C_7-C_{18} alkaryl; wherein, in formula (IV) each bond can represent either a peptide bond or a pseudopeptide bond, provided that said compound cannot contain more than 2 pseudopeptide bonds, or a pharmaceutically acceptable salt thereof.

13. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A^{29} and A^{30}.

14. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A^{30} and A^{31}.

15. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A^{31} and A^{32}.

16. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A^{32} and A^{33}.

17. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A^{34} and A^{35}.

18. The compound of claim 1, 5, 9, or 12, wherein a pseudopeptide bond is positioned between A^{35} and A^{36}.

19. A dimeric compound comprising one compound from either claims 1, 5, 9, or 12 and one compound from either claims 1, 5, 9, or 12, wherein said dimer is formed by either an amide bond or a disulfide bridge between the two compounds.

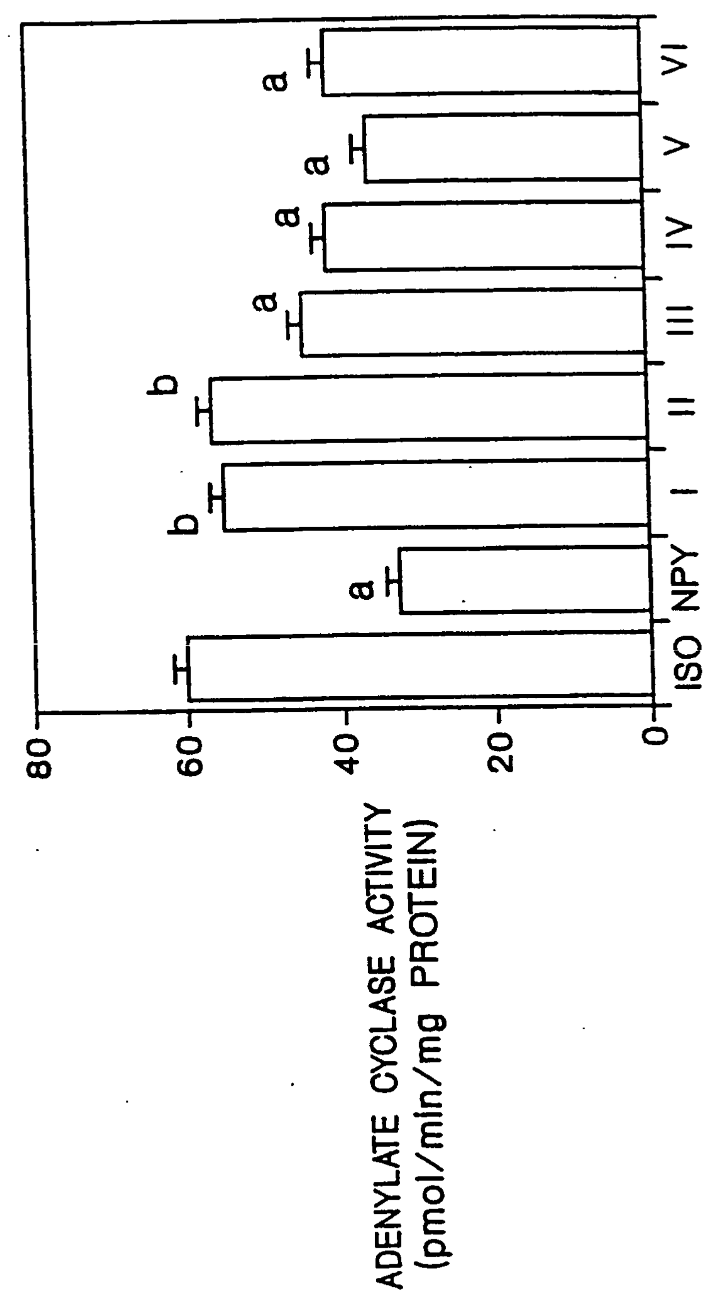

ADENYLATE CYCLASE ACTIVITY
(pmol/min/mg PROTEIN)
0
20
40
60
80
ISO
NPY
I
II
III
IV
V
VI
a
b
b
a
a
a
a

FIG. 1

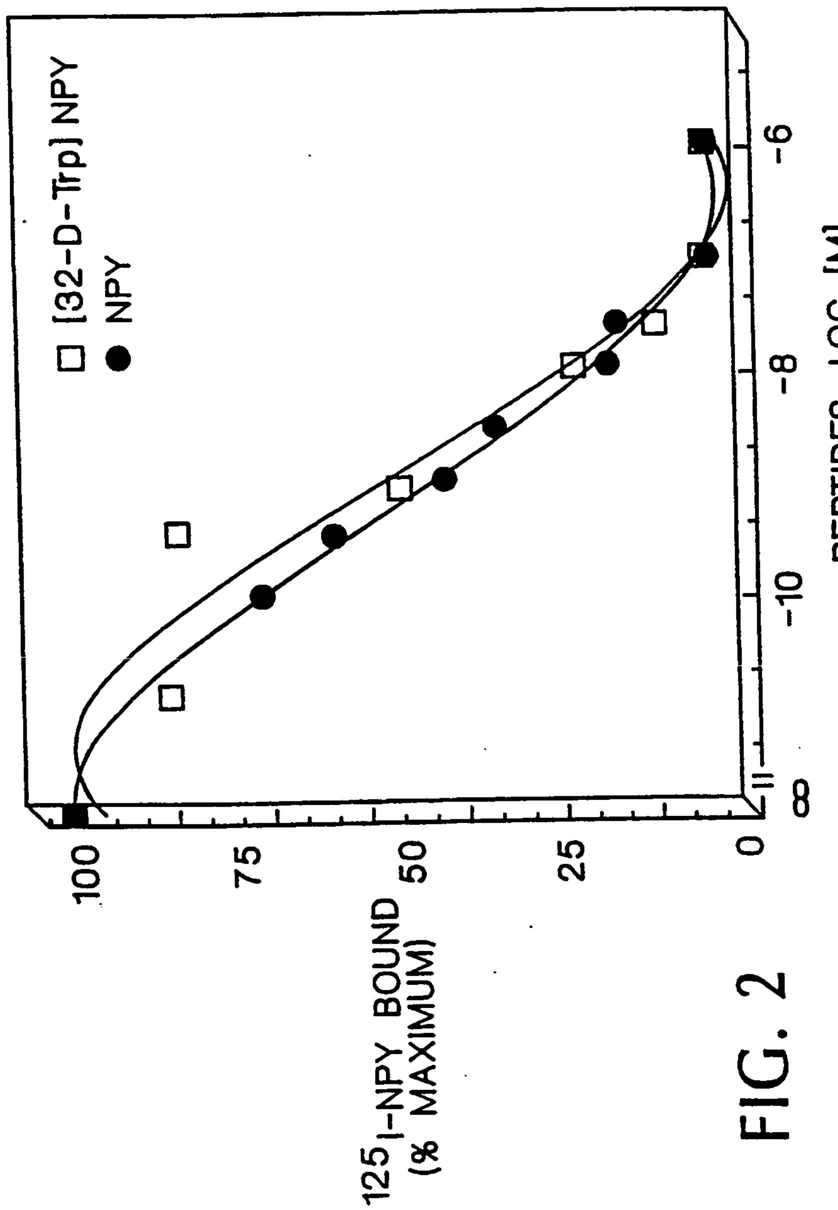

[32-D-Trp] NPY
NPY
^{125}I-NPY BOUND
(% MAXIMUM)
100
75
50
25
0
∞
-10
-8
-6
PEPTIDES, LOG [M]

FIG. 2

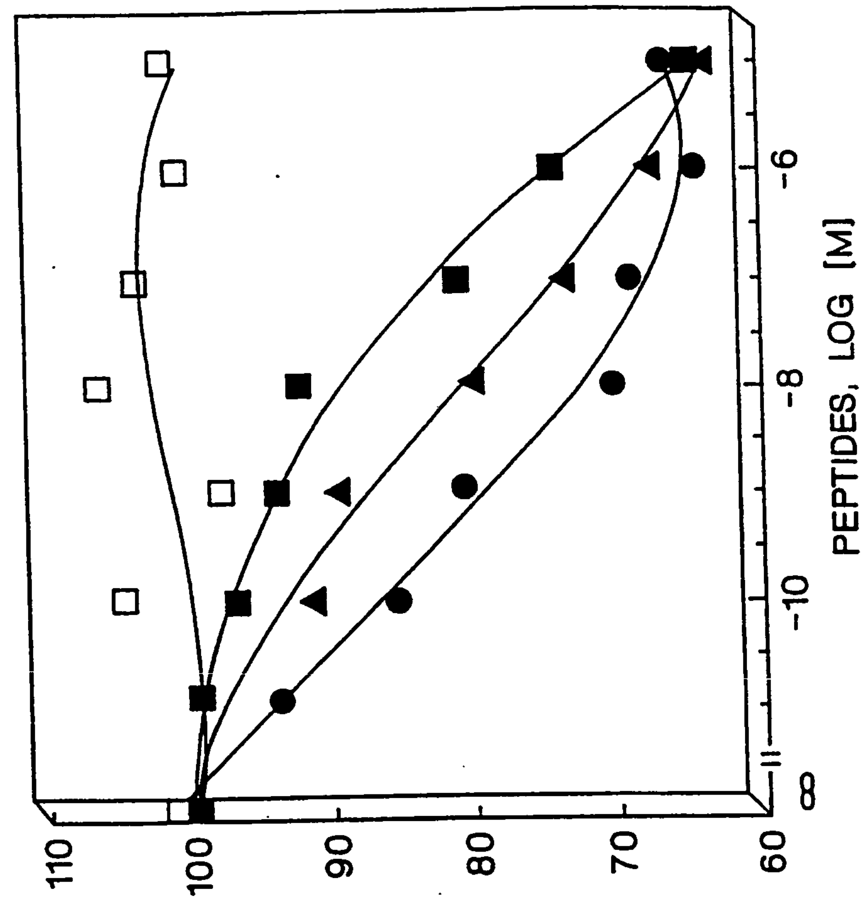
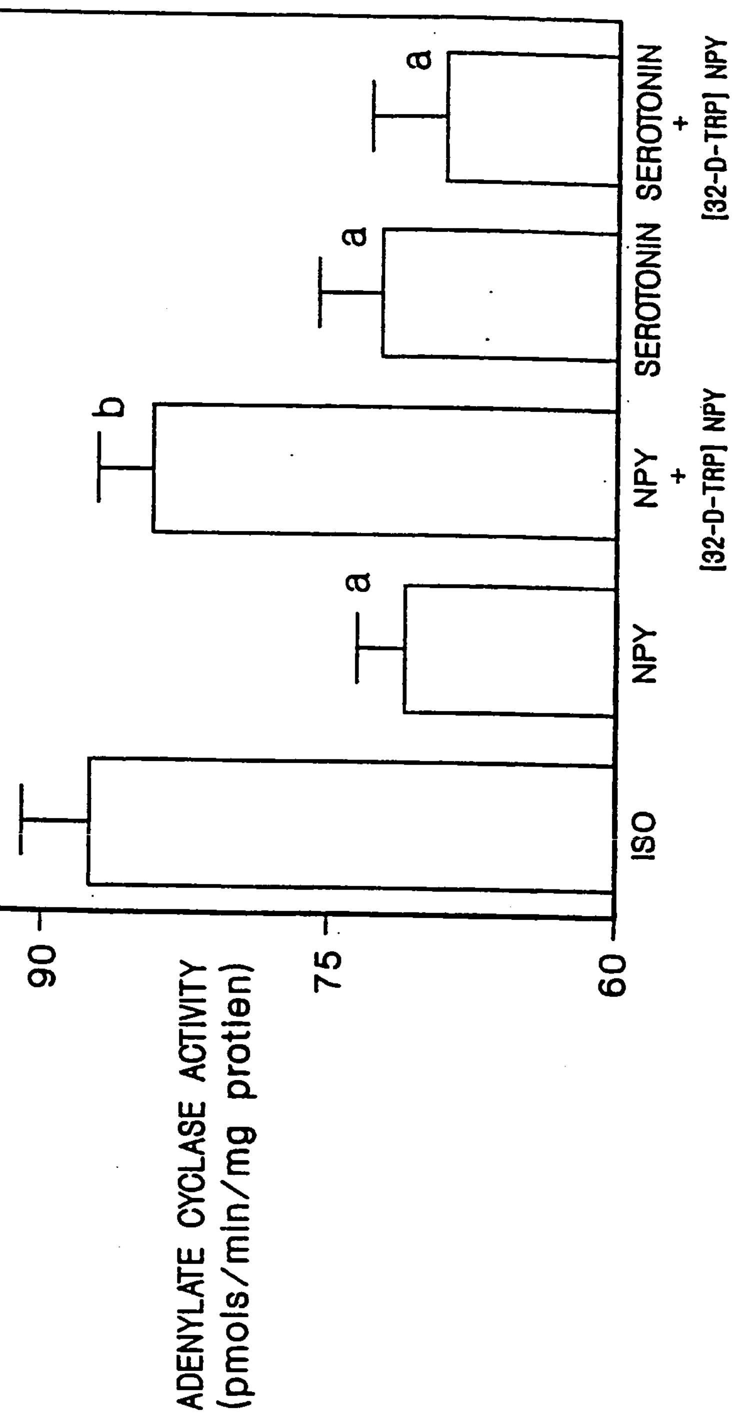

ADENYLATE
CYCLASE
ACTIVITY
(% MAXIMUM)
110
100
90
80
70
60
PEPTIDES, LOG [M]
-10
-8
-6

FIG. 3



ADENYLATE CYCLASE ACTIVITY (pmols/min/mg protien)
90
75
60
ISO
NPY
NPY + [32-D-TRP] NPY
SEROTONIN
SEROTONIN + [32-D-TRP] NPY
a
b
a
a

FIG. 4

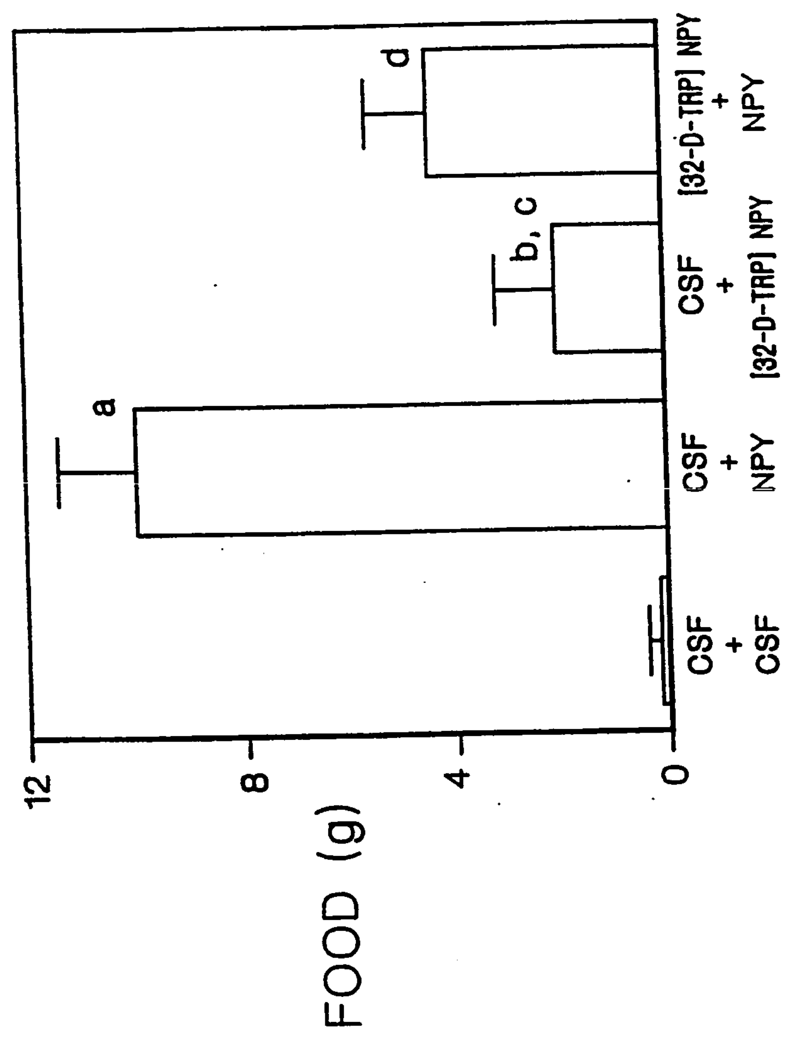

FOOD (g)
12
8
4
0
a
b, c
d
CSF + CSF
CSF + NPY
CSF + [32-D-TRP] NPY
[32-D-TRP] NPY + NPY

FIG. 5

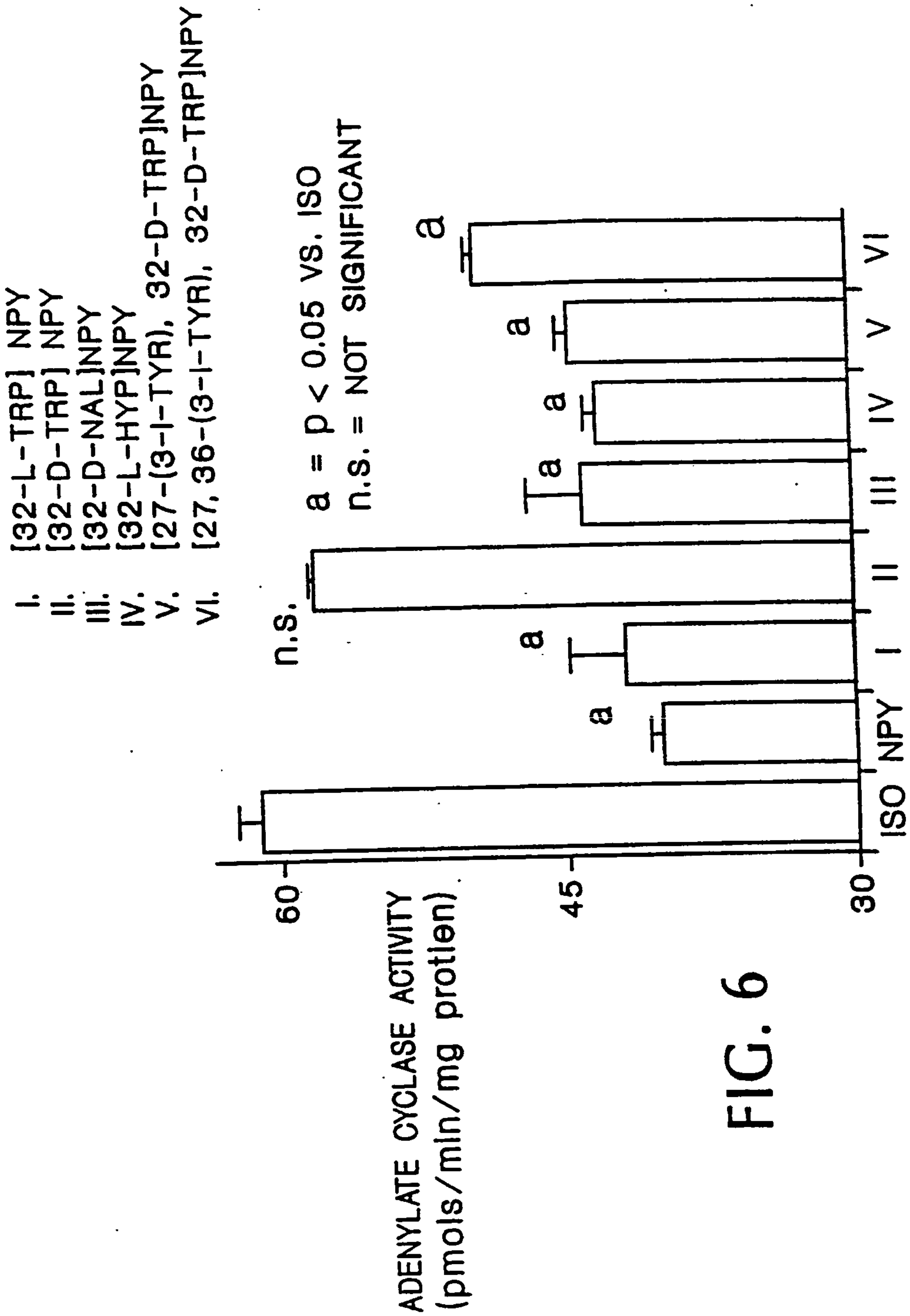

I. [32-L-TRP] NPY
II. [32-D-TRP] NPY
III. [32-D-NAL]NPY
IV. [32-L-HYP]NPY
V. [27-(3-I-TYR), 32-D-TRP]NPY
VI. [27, 36-(3-I-TYR), 32-D-TRP]NPY
a = p < 0.05 VS. ISO
n.s. = NOT SIGNIFICANT
ADENYLATE CYCLASE ACTIVITY
(pmols/min/mg protien)
60
45
30
ISO
NPY
I
II
III
IV
V
VI
a
a
n.s.
a
a
a
a

FIG. 6

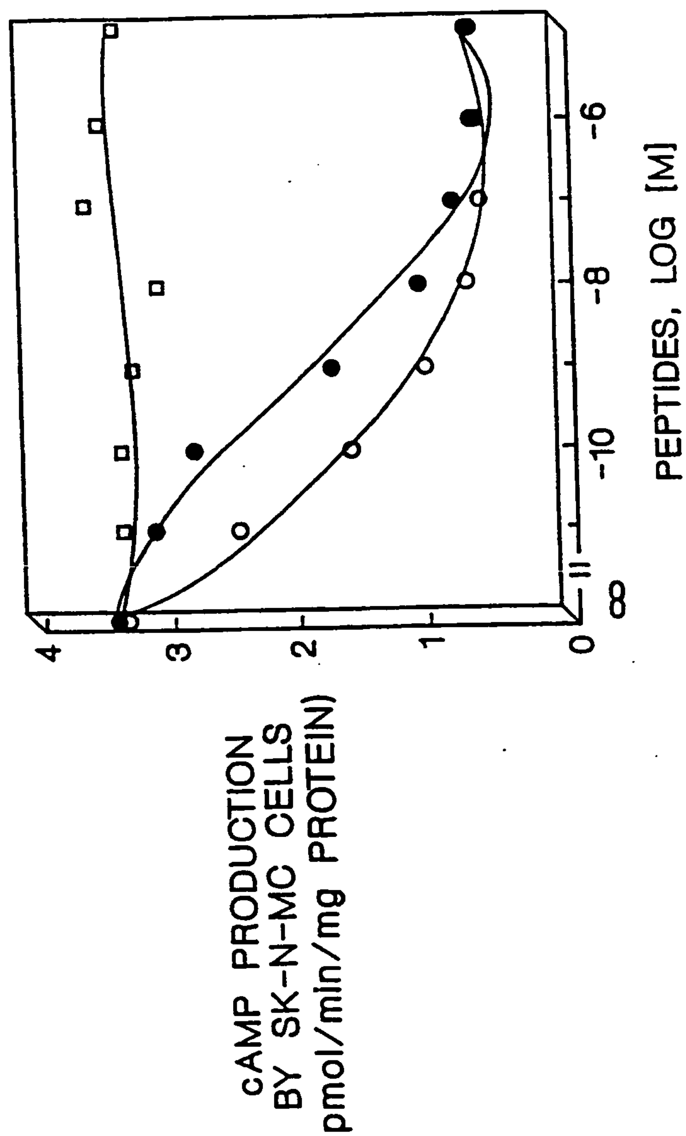

cAMP PRODUCTION
BY SK-N-MC CELLS
(pmol/min/mg PROTEIN)
0
1
2
3
4
PEPTIDES, LOG [M]
∞
-10
-8
-6

FIG. 7

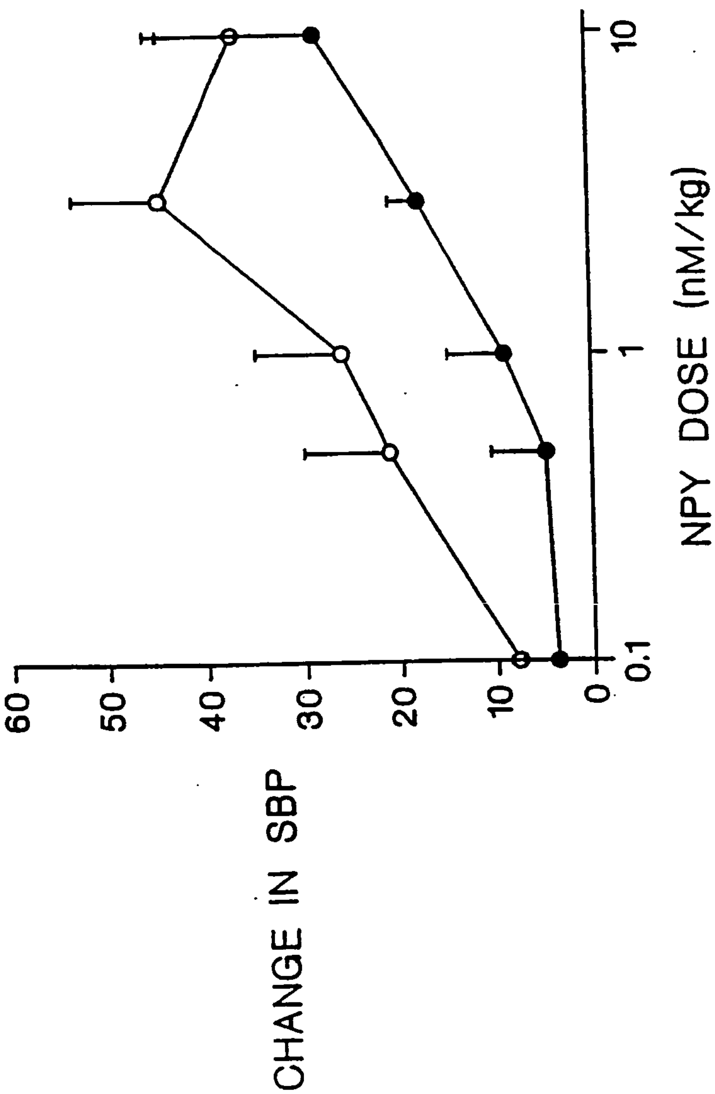


FIG. 8

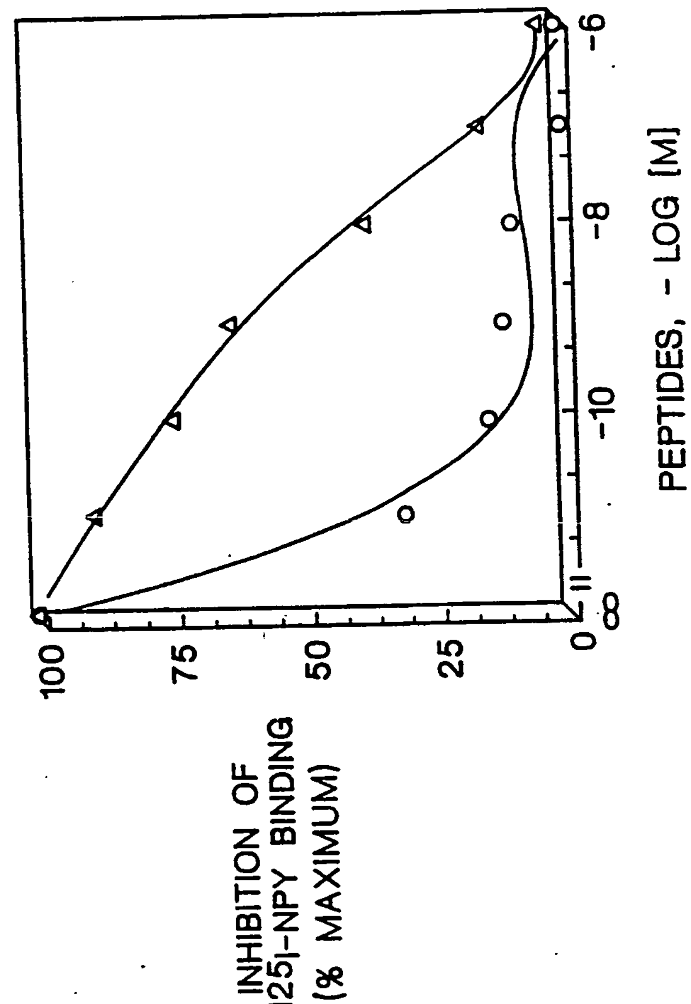

INHIBITION OF
125I-NPY BINDING
(% MAXIMUM)
100
75
50
25
0
∞
11
-10
-8
-6
PEPTIDES, - LOG [M]

FIG. 9

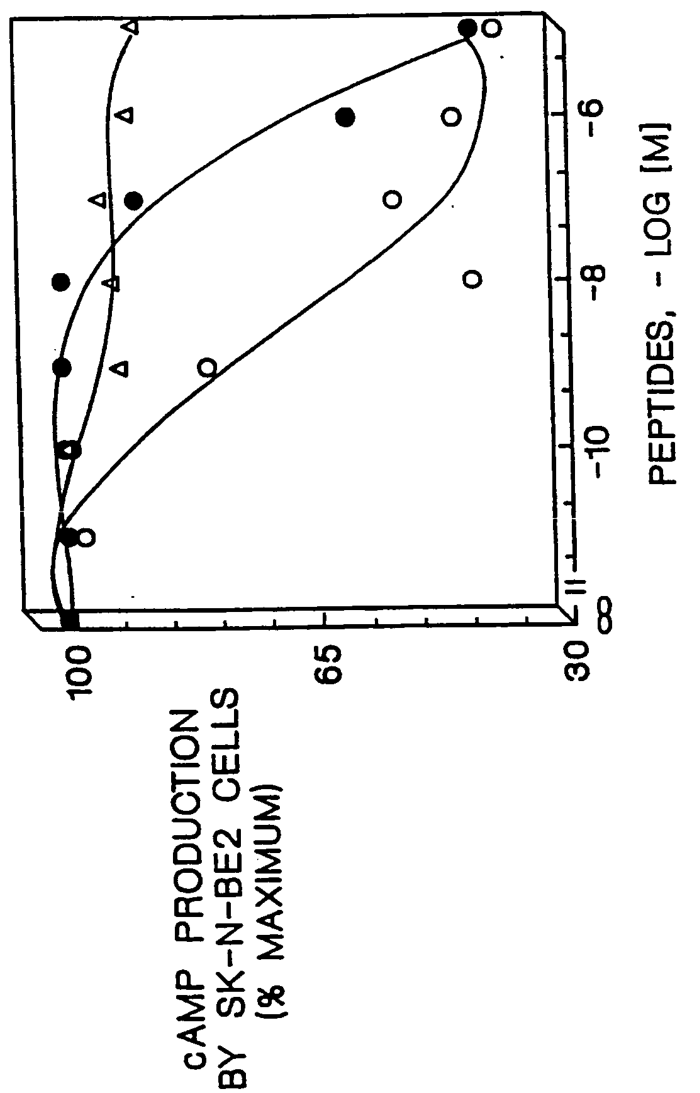

cAMP PRODUCTION
BY SK-N-BE2 CELLS
(% MAXIMUM)
100
65
30
∞
−10
−8
−6
PEPTIDES, - LOG [M]

FIG. 10

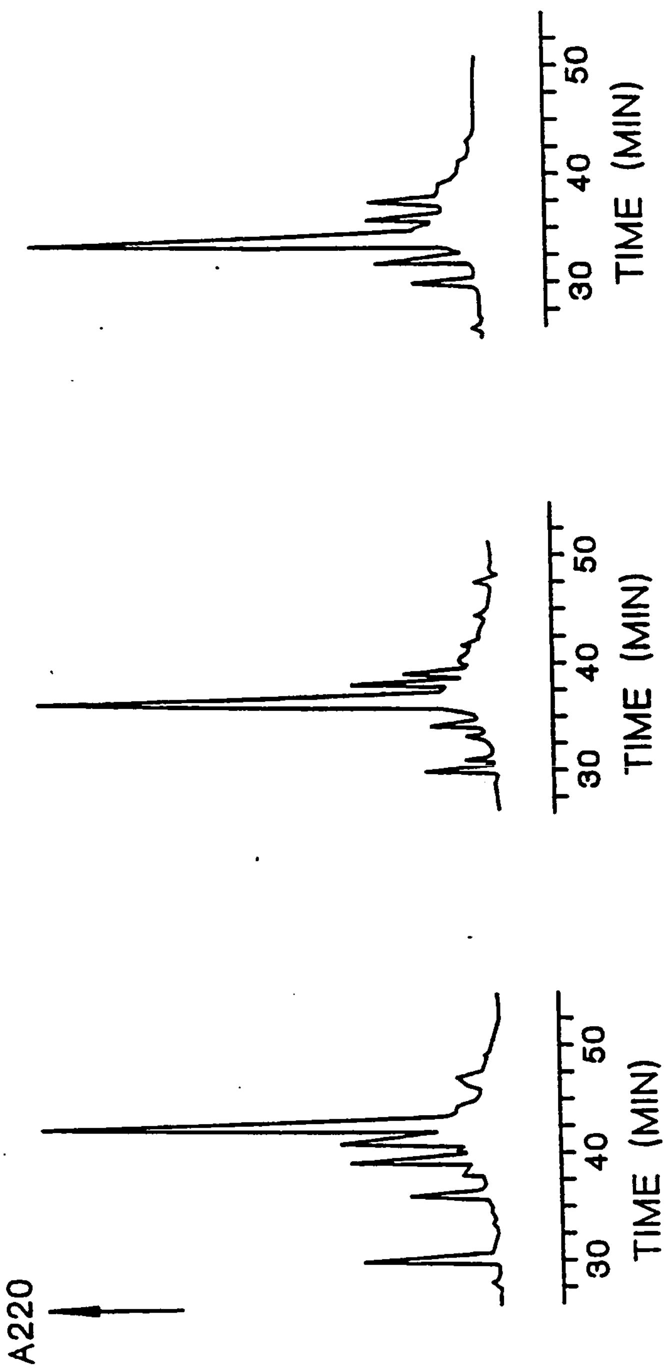


FIG. 11(a)

FIG. 11(b)

FIG. 11(c)

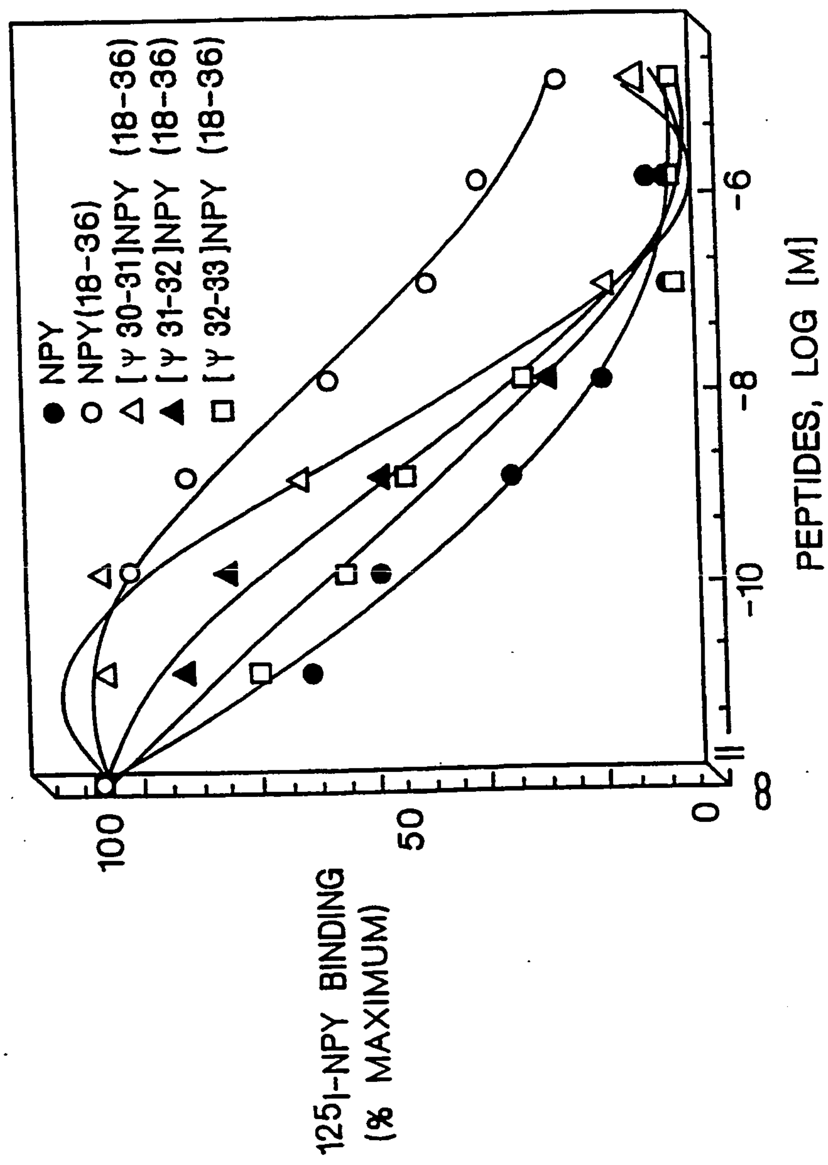

NPY
NPY(18-36)
[ψ 30-31]NPY (18-36)
[ψ 31-32]NPY (18-36)
[ψ 32-33]NPY (18-36)
100
50
0
∞
-10
-8
-6
PEPTIDES, LOG [M]
125I-NPY BINDING
(% MAXIMUM)

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06837

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02; C07K 5/00, 7/00, 15/00, 17/00
US CL :530/324, 325, 326
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

USPTO APS
search terms: neuropeptide Y

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,026,685 (BOUBLIK ET AL) 25 June 1991, col. 3, lines 20-35.	5, 6, 12
X	US, A, 4,839,343 (WAEBER ET AL) 13 June 1989, col. 1, lines 20-60.	1,9
X	EP, A,0,355,793 (KRSTENANSKY ET AL) 28 February 1990, see p. 2.	1,9
X	J. Med. Chem., Volume 36, Number 3, issued 1993, D. A. Kirby et al, "Defining Structural Requirements for Neuropeptide Y Receptors Using Truncated and Conformationally Restricted Analogs", pages 385-393, see compounds #4,5 and 17.	1,2,9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
08 AUGUST 1994	AUG 18 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	SHEELA J. HUFF Jill Warden for
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0916

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06837

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. Med. Chem., Volume 35, issued 1992, R. D. Feinstein et al, "Structural Requirements for Neuropeptide Y18-36-Evoked Hypotension: A Systemic Study", pages 2836-2843, see compounds #1-3, 6-9, 12-15, 19-20, 22-27.	12
X	J. Med. Chem., Volume 32, issued 1989, Boublik et al, "Synthesis and Hypertensive Activity of Neuropeptide Y Fragments and Analogues with Modified N- or C-Termini or D-Substitutions", pages 597-601, see compounds 1-3, 5-6, 9, 11, 20-21, 23-24.	1
X	J. Med. Chem., Volume 36, issued 1993, D. A. Kirby et al, "Neuropeptide Y: Y1 and Y2 Affinities of the Complete Series of Analogues with Single D-Residues Substitutions", pages 3802-3808, see Table 1.	1

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*